

## **REVIEW OF LITERATURE**

Pea (*Pisum sativum* L.) was the original model organism used in Mendel's discovery (1866) of the laws of inheritance, making it the foundation of modern plant genetics. A lot of research work regarding seed quality, seed and seedling vigor, line x tester crosses and other related parameters in pea is reported from most parts of the globe, but such information is readily available in India. Keeping in view, the vital importance of pea in India, the present investigation was carried out to study the main aspects of seed and seedling vigor and its impacts on overall performance of pea. However literature pertaining to present study is reviewed as under.

### **2.1 CONCEPT OF SEED QUALITY AND VIGOR**

Seed quality is a multifaceted concept comprising several components. Seed quality in agricultural production is a major factor to be considered in the deployment of a crop, so it becomes important to obtain information about seed vigor and germination. Planting of high quality seed at favorable conditions not only allows early and high quality products of high price, but also extends the harvesting period (Nienhuis et al., 1983). Seed quality is a limiting factor affecting, not only germination capacity but also emergence potential, field stand and uniformity, seedling growth and finally crop productivity. Seed quality (seed viability and vigor) has a profound effect on seed performance, stand establishment and ultimately economical yield. Harrington (1971) and Ware and McCallum (1975) specified the main characteristics that constitute seed quality and play a major role in success or failure of the crop. These include genetic purity, physical purity and freeness from insect pests and diseases.

The development of a satisfactory definition of seed vigor has been a central theme in the development of vigor tests. Definitions of seed vigor have evolved over the years due to the challenge in finding one description that incorporates all vigor characteristics. The earliest records of the seed vigor dates back to 1876, when Nobbe reported on 'germination energy'; introduced the term 'triebkraft' meaning 'driving force' or 'shooting strength' in his Handbuch der Samendurde (Berlin). The term "vigor" was then used by a German Plant Pathologist, Hiltner, who observed that cereal seeds infected by Fusarium disease were capable of germination but the resulting seedling were incapable of penetrating through a thick layer of Ziegelgrus (brick grit) under a specified conditions of temperature and moisture (Hiltner and

Ihssen,1911). He called this ability as “Triebkraft” meaning shoot strength and “driving force”. Later on it was translated into French as “Vigueur” and in English as “Vigor”. The brick grit test was adopted in Germany to indicate the seedling vigor (Eggebrecht,). Isley (1957) defined vigor as “the sum total of all seed attributes which favor rapid and uniform stand establishment in the field”. Delouche and Caldwell (1960) modified Isley’s definition of seed vigor to “the sum total of all seed attributes which favor rapid and uniform stand establishment in the field.” Woodstock (1969) stated that “vigor” is the condition of active good health and natural robustness in seeds, which upon planting permits germination to process rapidly under a wide range of field conditions.

Makashera (1973) stated that “Vigor” is the condition of active good health and natural robustness in seed which upon planting permits germination to process rapidly under a wide range of experimental conditions. Gelmond et al. (1978) defined seed vigor as potential ability of seeds to yield the maximum plant products at the earliest time under variable environmental field conditions. Bishnoi and Delouche (1980) stated that seed vigor is an aspect of seed quality which control field stand establishment and vigor tests are required to obtain reliable assessments of field performance. Seed vigor as per Sundstrom et al. (1986) is the capacity of a seed lot to germinate quickly and completely with subsequent uniform seedling development. Seshu et al. (1987), outlined vigor as a concept usually derived from the observation of differences between seed lots, and a wide definition based on the effect have to be considered more appropriate than one based on causes. Chin (1988) defined seed vigor as the sum total of those properties of seeds which determines the level of activities and performances of seed lots during germination and seedling emergences.

Seed vigor definition as adopted by AOSA (1983) “Seed vigor comprises of those seed properties, which determine the potential for rapid, uniform emergence and development of normal seedling under a wide range of field conditions”. International Seed Testing Association, ISTA (1987) defined seed vigor as the sum total of those properties of the seed that determine the level of activity and performance of the seed during germination and seedling emergence. Later International Seed Testing Association, ISTA (1995) expanded the previous definition and defined vigor as the sum total of those properties of seeds which determines the level of activities and performances of seed lots during germination and seedling emergence.

Seed vigor is a measure of the extent of damage that accumulates as viability declines. Seed vigor had become very important because it is more closely related to field emergence under stress conditions than the standard germination test (Tekrony and Egli, 1977). Association of Official Seed Analysts (2009) states that vigor test is absolute to predict field emergence of a seed lot that can not be though assessed by an ordinary germination test in laboratory.

The concept and importance of high vigor seeds has been well understood. Considerable researches have been done and still continuing to acquire knowledge into better understanding of seed vigor. Several approaches have been made to identify vigor of wheat (Modarresi et al., 2002); soybean (Torres et al., 2004); corn (Singhabumrung and Juntakool, 2004); pumpkin (Dutra and Vieira, 2006), kale (Komba et al., 2006) and pea (Hampton et al., 2004; Ishrat et al., 2012 (A)) etc.

## **2.2 SEEDLING VIGOR**

In the literature seedling vigor is a complex concept that includes many characteristics contributing to successful development and growth in the early seedling stages. Seedling vigor is the ability that enables a seedling upon germination to grow rapidly and get established in shortest period of time. It is related to all phases of seedling development from seed germination up to stand establishment in field. Whalley et al., (1966) subdivided seedling growth into heterotrophic stage, a transition stage and an autotrophic stage, which occurs after all endosperm reserves have been depleted. Heydecker (1972) used the term seedling vigor to indicate the overall ability of seed to emerge its aerial parts from the soil/water in a suboptimal environment. Glewen and Vogal (1984) stated that seedling vigor is the combination of all components of seedling growth.

Lee et al., (1986) defined seedling vigor as a quality factor that denotes the potential for rapid germination and faster seedling growth under field conditions and that this potential varies according with the genetic and environmental backgrounds. The performance of genotypes at germination, heterotrophic growth, and early autotrophic growth is collectively referred to as seedling vigor and early vigor (Revilla et al., 1999; Hund et al., 2004). The uniform germination and consequent normal growth of seedlings is important to disease protection and yield as well as the quality of the crop (Makoto et al., 2012).

According to Birendra et al., (2012) seedling vigor is the property of the seed that enable it to germinate rapidly and vigorously under favorable conditions in the laboratory and such seedlings are likely to give effective stand in the field. The early growth of seedlings is crucial for their establishment and hence for their eventual success in terms of biomass production or seed output (Kuldeep et al., 2011).

### **2.3 SEED AND SEEDLING VIGOR EVALUATION**

There are a number of tests recommended by various agencies to measure seed and seedling vigor. Frank (1950) proposed the name “seedling vigor test” to any test designated to produce the results similar to those occur in the soil. Hydecker (1969) stated that a successful vigor test should be cheap, repeatable, easy to perform and rapid. It should give reproducible results that can be objectively assessed, clearly recorded and meaningfully interpreted.

Simple traits such as seedling height, seedling dry weight and kernel/seed weight (Regan et al., 1992; Trachsel et al., 2010), rapid shoot growth, shoot dry weight and shoot length (Sasahara et al., 1986; Grando & Ceccarelli 1995) have been identified as good indicators of good seedling vigor in barley. Mark (2011) stated that seed vigor cannot be directly assayed (as germination) with results expressed in absolute terms. There may be minor differences in the testing environment (e.g. temperature, initial seed moisture, test duration) that can cause major changes in test results. The general strategy of determining seed vigor is to measure some aspects of seed deterioration or weakness, which is inversely proportional to seed vigor.

Miller (2010) described that it is too much difficult to interpret the actual field performance of a seed lot on the basis of a single seed vigor test, because the stress conditions are seldom uniform throughout the year and varies from one agro climatic zone to another. Since a single test does not necessarily measure all aspects of seed vigor, several tests have been suggested (Hampton and Coolbear, 1990). The International Seed Testing Association (1995), also recommended that several tests should be used, because no single test satisfies all the requirements of seed vigor.

### **2.4 FACTORS AFFECTING SEED AND SEEDLING VIGOR**

There are various factors which are responsible for the differences among seed and seedling vigor. Perry (1980) identified the possible causes of seed vigor differences and stated that vigor is determined by the genotype but it may be modified by the environment during seed maturation, pre and post harvest handling and storage.

Perry (1980) listed the major factors which may induce variation in seed quality, as: genetic constitution, environment and nutrition of the mother plant, stage of the maturity at harvest, seed size and weight, seed position on the mother plant, mechanical integrity, deterioration and aging.

Brocklehurst (1985) listed the principal factors ,which influence seed quality throughout the life of the seed, from the time of fertilization on the mother plant until the moment of seeding, as: seed genotype, environmental conditions during seed development, seed position on the mother plant, harvesting timing and techniques, storage conditions and pre-sowing treatments. There are other factors which affect seed vigor such as genetic make-up, mother plant nutrition, field weathering, time and method of harvesting, drying, processing, treating and storage as well as time of sowing and harvest (Adetunji, 1991; Castillo, Hampton and Coolbear, 1994).

Basu (1995) and Mahajan et al., (1998) reported the factors influencing seed quality as: preharvest conditions that includes, quality of the initial seed, soil fertility, temperature and photoperiod, moisture status of soil and pesticide or herbicide application; harvesting and processing conditions and post-harvest storage conditions including: seed moisture and drying, storage temperature and oxygen pressure. Some important factors which effect seed vigor are explained as under:

#### **2.4.1 Seed Size**

In pea, large seeds gave rise to taller plants which flowered earlier (Kant et al., 1973). Townsend (1974) stated that positive correlation usually occurred among the traits such as seed size, seedling vigor and seedling emergence in forages and that selection for larger seeded progenies with relatively rapid seedling emergence would be effective. Mckersie and Tomes (1982) estimated seed quality, seedling vigor and field establishment in three forage legumes and found that seed lots with poor germination generally had poor seedling vigor and seed weight did not showed any significant correlation with field establishment.

Arain (1990) reported 25-50 percent higher seedling vigor, viability and germination in high density grains than low density grains in 10 rice cultivars. As per finding of Amin (1999), mungbean seed size showed no significant effect on yield except large seeded plants flowered and matured earlier than small seeded plants producing heavier seeds. In Maize, large seeds were observed to have maximum emergence per area, maximum plants height, maximum number of plants per area and low mortality percent (Khan et al., 2005). Similar trend was observed by Na-

Chiangmai et al. (2007) where large mungbean seeds had advantages only in sprout production than small seeds without any effect on final yield.

Khan et al., (2006) studied the prevalence of seed borne fungi and seed vigor on the basis of physical appearance of seed and concluded that apparently big healthy seeds had higher germination and improved seedling vigor (shoot and root length).

Ishrat et al., (2009) observed a positive correlation between seed size and seedling vigor in rice. Anuradha et al., (2009) revealed that the seed size had a positive association with seed quality parameters in chick pea. They found that larger size seeds retained on 19/64" round perforated metal sieves recorded the maximum germination and seedling vigor. The protein content, dehydrogenase activity and -amylase activity were also more in larger sized seeds followed by medium sized seeds.

#### **2.4.2 Seed maturity and stage of harvesting**

Maximum seed viability and seed vigor may be achieved if seeds are harvested at the correct stage of maturity. Physiological maturity, (mass maturity) and harvest maturity are two important terms which are closely related to seed quality. While, it is desirable to harvest the seed when possible at the physiological maturity, but, practically, one must wait till harvestable maturity, when seed moisture content is sufficient low to permit mechanical harvest with no damage to the seed. Harvesting of seed crop at appropriate stage is important to achieve high seed vigor (Austin, 1972; Copeland and McDonald, 1995).

Matthews et al (1980) reported that harvesting immature pea seeds containing high moisture level is a cause of the production of low-vigor seeds and more physical and mechanical damage occurred to their soft testa in terms of coat cracks during harvest and processing operations, facilitating rapid imbibition and leakage. Under storage these seeds deteriorated rapidly.

Ellis et al., (1987) have reported that maximum seed quality and vigor in peas, chick peas and lupin was found to be reached at physiological maturity, at 45% moisture contents in soybean and 30% moisture in faba bean and lentil. It has been reported that maximum seed quality (vigor) does not occur until some time after physiological maturity (Ellis and Pieta-Filho, 1992; Zanakis et al., 1994).

According to Chuntarachurd et al. (1984) yard long bean seeds harvested at 16 days after pollination produced highest seed weight and seed vigor. Khattrra et al. (1997) reported that in pea, the maximum physiological maturity acquired depends on

the cultivar. They also found that maximum dry weight, germination potential and seed vigor as measured by root and shoot length and seedling dry weight were attained in the 35 and 46 days after anthesis.

Hu et al., (1999) reported low viability and vigor in rice seeds matured in high temperature. In common bean, maximum viability was achieved at moisture contents of 31-37% which was well beyond physiological maturity but maximum seed vigor was archived at physiological maturity (Muasya et al., 2002).

Sachan et al., (2003) revealed that stage of harvesting had a direct effect on quality of pea seeds. They concluded that highest mean germination percentage as well as average vigor index was recorded in all the varieties at 40 days after anthesis, and these quality parameters showed a sharp decline, when the seeds were harvested 50 days after anthesis.

### **2.4.3 Mechanical injury and ageing**

Mechanical injury during post harvest operations such as threshing, cleaning, handling and planting is considered as one of the most important factors influencing quality of seed and thus seed and seedling vigor. Care must be taken to avoid any damage to the seed during seed handling operations. The seeds harvested by mechanical harvesters are more prone to seed coat damages as compared to hand harvested ones, thereby deteriorating at a faster rate due to entry of chemicals and herbicides into embryo eventually leading to death. Toole and Toole (1960) found that the viability of stored garden bean seed decreases more rapidly in lots with the greatest mechanical damage.

Studies in bean and cabbage seeds showed that the external damages were much larger in bigger bean seeds than in smaller cabbage and the mechanical abuse was not only visible (such as cracked seed coats and broked seeds in both species) but also invisible, internal injuries, which caused lower germination down to 60-70% and increased the number of abnormal seedlings. In cotton, handpicked and laboratory ginned lots were of better quality than mechanically harvested and commercial ginned seeds (Bishnoi and Delouche, 1980).

During aging decline in seed vigor, respiration rate, and sugar content accompanied by a complete declined of alpha amylase activity are noticeable. Decrease of phospholipids, carbohydrates, sugars, proteins and peroxidase activity with increased fatty acids were observed during aging of corn seeds (Basavarajappa et al., 1991). Iqbal and Smith (1996) stated that pea seed germination percentage, field

emergence percentage, root and shoot length and dry matter content were reduced and abnormal seedlings and electrical conductivity become higher with increasing severity of aging.

Larson et al., (1998) demonstrated that seedlings produced from the older pea seed lots emerged more slowly than seedlings from the younger seed lots, the differences in the growth attributes diminished through out the growing season. Rise in respiration rate, phosphatase activity and sugar content accompanied by a complete decline of alpha amylase activity and RNA, DNA and protein content were noticeable in rice during seed deterioration. (Zhoe et al., 2002).

Pandita and Shanta (2002) conducted an experiment to study the effect of seed ageing on vigor of pea seeds. At the end of their studies they found that laboratory germination, speed of germination, and vigor were significantly reduced from 97.5 to 87.0%, 21.0 to 15.8, and 1650 to 1164, respectively, as the aging process progressed from 8 to 32 months. The conductivity of seed leachates increased with the aging process (16.7 to 24.3 m Siemens/seed) during the same period of time.

#### **2.4.4 Environment during seed production**

Little information is available concerning the effect of environmental factors on seed quality. A complex of environmental factors acting during development and maturation of the seed are related to subsequent germination and vigor. Peacock and Hawkins (1970) studied the effect of seed moisture on seedling vigor and other agronomic characters in cotton and reported that seeds produced in higher temperature and low rainfall conditions resulted in the poorer seedling vigor and low yield. However, Nichols et al, (1978) working with potted peas plants observed no effect of drought stress on seed conductivity or germination. In peas, maturation in cool temperatures led to the development of larger seeds which gave rise to taller plants and earlier flowering in comparison to those of the same varieties which had matured in warmer temperatures (Kant et al., 1973).

Teckrony et al., (1980) reported the reduction of yield, viability and vigor in soybean by environmental stress. Smiciklas et al., (1989) mentioned that drought stress could lower seed quality and germination of seeds of soybean if applied during various stages of flowering and seed development.

Environmental stress during seed production can affect subsequent seed quality. Stress occurring after physiological maturity, but before harvest can cause reduction in soybean seed germination and vigor (Tekrony et al., 1980; Vieira et al.,

1991 & 1992). In soybean, Spears et al. (1997) revealed that high temperature during seed development reduced seed vigor in the absence of associated mechanical injury and seed borne diseases. Ojo and Ariyo (2000) studied change in seed quality during seed development and maturation among six genotypes of soybean from four harvesting dates in three contrasting ecologies under rainfed conditions. They revealed that quality of seeds obtained from each of the three sites differed substantially. The combined analysis of variance of percentage germination and seedling emergency showed wide variations among genotypes, locations, harvesting time and their interactions probably due to differences in climatic conditions and soil type from one site to another.

Khalil et al. (2001) found that seed germination was inversely proportional to temperature experience by mother plant during pod filling stage which resulted into decrease in germination by 10.7% with 1% increase in temperature in soybean seeds. Waldron et al. (2006) found three different environmental conditions had little effect on seed yield and seedling vigor. According to Woltz et al. (2006) freezing temperatures reduced germination and vigor of corn seeds and to obtain higher germination and vigor, the corn seeds should be harvested immature stage before the freezing event. Likewise Wang et al., (2006) also revealed that high temperature stress during pod development decreased pod fertility, seed set and seed yield in chick pea.

#### **2.4.5 Genetic variability**

Differences in seed vigor usually arise as a result of various factors which effect seeds. Genetic differences in ability to germinate at suboptimal temperatures in peas (Torfason and Nonnecke, 1959) have been reported. A higher incidence of hollow heart has been found in wrinkled than unwrinkled seeded peas, which means the former possess lower seed vigor than unwrinkled cultivars (Perry, 1980). White seeded cultivars of dwarf bean, and lighter seeded types of chickpea were found to have high rates of water uptake and therefore a high incidence of imbibition damage, leading to lowered vigor and poor field emergence (Powell et al., 1986). Varietal differences in the minimum germination temperature of four cucumber cultivars were recorded by Kapitsimadi et al., (1990).

Shukla et al., (1994) reported variation in seed vigor (seedling dry weight) and germination percentage after 8th day among garden pea cultivars. Kumar et al. (1997) stated that pea seed longevity is genotypically controlled. Seed germination

and seedling vigor, depending on cultivar/genotype, decreased with increasing storage period at 80% RH and 40°C (Horcika and Hosnedi, 1997). They found that seed size within genotype (cultivar) had an affect on seed longevity in some cultivars. The vigor differences in pea in terms of electrical conductivity values were found to be varying from one cultivar to another.

Taweekul et al., (1998) recorded that high vigor pea seed lots emerged well under wet and cold soil conditions (more than 90 %). In contrast, low vigor seed lots, depending on cultivar/genotype and vigor status, emerged poorly and slowly (43-62%) resulting in lower plant establishment, low leaf area index, and leaf area duration. According to Ali et al., (2003), germination of rice varieties in low temperature was influenced more by genotype than seed quality.

Bhupendra and Gangwar (2005) evaluated genetically pure seeds of several mungbean cultivars and revealed that all the cultivars differed in their seed vigor index, and seed volume weight also differed significantly in most vigor parameters. Finding of Atak et al. (2008) also revealed that dark green colored pea seeds germinated faster had highest shoot, root length and fresh weight resulting into higher seed vigor than light and medium colored seeds.

## **2.5 CLASSIFICATION OF VIGOR TESTS**

Seed vigor test is more sensitive index of seed quality than germination test, any of the events which precede loss of germination could serve as vigor tests. Different systems of classification have been proposed to categorize the vigor testing procedures. According to Isley (1957) there are two types of vigor tests i.e., direct test and indirect test. Direct test are based on the interaction of seed with the environment and measure the ability of seeds to emerge under simulated field stress conditions e.g. the cold tests. Indirect tests measure some physiological characteristics of the seed such as electrical conductivity test. Woodstock (1973) has divided these tests into physiological and biochemical tests.

McDonald (1977) divided vigor tests into three main groups: a) Physical tests that measure seed characteristics such as seed size or seed density; b) Physiological tests that utilize parameters of germination or growth and c) Biochemical tests that monitor the chemical reactions involved in cellular maintenance such as tetrazolium test. Other invigorators have classified these tests into stress and quick test groups (Pollock and Roos, 1972).

AOSA (1983) classified vigor tests into a) biochemical test such as tetrazolium (TZ), conductivity, respiration, glutamic acid decarboxylase activity (GADA) and ATP content, b) stress test such as cold test, accelerated aging, cool germination, brick test and osmotic stress and c) seedling growth and evaluation tests such as seedling vigor classification, seedling growth rate, speed of germination.

### 2.5.1 Physical Tests

These tests are based on the physical seed characters such as seed size, weight and density. These are cheap and quick ones and can be conducted on a large scale and show positive relationship with vigor (McDonald, 1975). West and Harris (1963) used seed coat color as criteria for vigor testing for Alfalfa. Peiffer et al., (1972) suggested the rate of imbibition as a vigor test and proposed that seed which require one hour of immersion in water are inferior to those which need 24 hours to imbibe water. Smith et al., (1973) used physical parameters such as seed thickness and weight to determine the appropriate parameters which could be used to separate higher vigor seeds from the lower ones in lettuce.

### 2.5.2 Physiological Tests

Physiological tests measure some aspects of germination or seedling growth. Gassim (1986) recommended two types of physiological tests a) Those carried under favorable environmental conditions e.g., speed of germination and seedling growth rate and b) Those carried out under unfavorable conditions such as accelerated ageing and brick grit test.

#### 2.5.2.1 Speed of germination:

Speed of germination is considered as an important vigor aspect because it provides a reasonably good index of vigor status of a seed. According to Pollock and Roos (1972) most of vegetable seed growers use the first counts of germination tests to evaluate vigor among the seed lots. Copeland (1976) reported two formulae for measuring coefficient of germination (C.G.) and vigor index. A faster germination rate will facilitate early seedling establishment (Seshu et al., 1987). Seed lots with similar total germination often vary in their rate of germination and growth. Unlike in standard germination test, the normal seedlings are evaluated on daily basis starting from first count till the final count and are calculated as follows:

$$\text{Speed of germination} = \frac{\text{Number of normal seedlings}}{\dots\dots\dots} + \dots\dots\dots + \frac{\text{Number of normal seedlings}}{\dots\dots\dots}$$

It is assumed that the higher the number, the higher is the seed vigor. The advantage of the speed of germination test is that very little work is required when compared to the standard germination test.

#### **2.5.2.2. Germination percentage test:**

A germination percentage test gives estimation about field survival of progeny plants from the seed and results can be used to differentiate between different seed lots. The standard germination test is conducted under controlled laboratory conditions. Hence there is no guarantee that the seeds will also give almost the same emergence percentage in the field. Germination is a complex process during which the imbibed mature seed must quickly shift from a maturation- to a germination-driven program of development and prepare for seedling growth (Nonogaki et al., 2010).

In a laboratory test, seed germination is the emergence and development of a seedling to a stage where the aspect of its essential structures (root system, shoot axis, cotyledons, terminal buds) indicates whether it is able to develop further into a satisfactory plant under favorable soil conditions (ISTA 2010). The categorization of normal and abnormal seedlings is based on internationally agreed definitions. Normal seedlings are those seedlings which are capable of becoming a normal plant in the field, comprising at least one main root and a normal shoot. Abnormal seedlings in contrast are not able to develop into a normal plant in the field because one or more essential structures are missing, e.g. no main root, no coleoptile, broken mesocotyl, deformed small seedlings. There are standards of this evaluation according to species, in which essential structures for normal and abnormal seedlings are prescribed. For Peas (*Pisum sativum* L.) sand or double filter paper is recommended as the germination substrate. The temperature during the test is 20°C, while no light is necessary. The first and final count days are the 5th and 8th day of the test. For fresh and dormancy seeds diffusion of light into the seeds is recommended.

#### **2.5.2.3 Seedling growth rate**

This is the oldest test for evaluating seed and seedling vigor. Seedling growth rate is measured in different ways such as measuring seedling height at intervals, counting the number of vigorous seedlings and seedling weight (Perry, 1973). According to ISTA (1995), the seedling growth rate test is based on the concept of accumulation of synthesis and accumulation of new materials and their rapid transfer

to the emerging embryonic axis, resulting in increased vigor. The vigor results are expressed as mg/seedling. Seeds are germinated according to the standard germination test but kept in the dark germinator. After 7 days normal seedlings with cotyledons or endosperms removed are oven dried at 80°C for 24 hrs and weight to determined seedling dry weight. The test has been suggested and recommended in corn and soybean seeds (AOSA, 1983; ISTA, 1995). Vanzolini et al., (2007) recommended seedling length as the accurate method of determination of vigor in soybean.

#### **2.5.2.4 Seedling vigor classification**

This vigor test is similar to the standard germination test. The only difference between the two tests is that normal seedlings are further classified as “strong” and “weak”. The basis for the test is that the “strong” and “weak” classification provides means of separating seedlings free of deficiencies from those with deficiencies which are symptomatic of low vigor or reduced quality. The test has been successfully used and recommended in beans, soybean (AOSA, 1983; ISTA, 1995), peanut and cotton (AOSA, 1983) and pea (ISTA, 1995).

#### **2.5.2.5 Cold test**

The most widely used vigor test which imposes stress on germinating seedlings is the cold test. It is a germination test that starts in cold soil and is completed in warm soil. This test was first developed at Iowa State University (Isley, 1950). In this test seeds are exposed to cold temperatures (10°C for 7 days) in non-sterile field soil at approximately 60-70% of water holding capacity, followed by 4-7 day grow out period in ideal conditions (25°C). The moisture and temperature provided in the cold test simulate the adverse conditions that seeds might encounter in an early spring planting. Besides this two stress conditions, other factors like seed quality, genotype, pathogens and seed treatment may also affect seed vigor. When germination obtained in the cold test is very close to that obtained in the standard germination test, the seed lot is expected to be vigorous (ISTA, 1995).

The cold test has been traditionally used for vigor testing of cotton (Bishnoi and Delouche, 1980), sorghum (Hampton, 1994) and is also recommended for maize (AOSA, 1983; ISTA, 1995). Miguel and Cicero (B) (1999) studied cold test for evaluating bean seed vigor. Vieira et al., (2010) recommended cold test as the best method of detecting vigor of soybean seeds.

### **2.5.2.6 Accelerated aging test**

Accelerated Ageing (AA) was initially developed as a test to estimate the longevity of seed in commercial storage and has been used to predict the life span of a number of different species. Delouche and Baskin (1973) developed accelerated aging test procedure to measure seed storability and evaluate vigor. The seeds undergo a stress of high temperature (40-45°C) and high relative humidity (100%) for a particular period (2-8 days depending on the species). After the ageing period, which may be different for different crop species, the seed is planted in germination test conditions. The closer the AA number is to the germination the better the vigor. Results are expressed as a percentage normal germination after ageing (vigorous seedlings). High vigor seeds can tolerate the aging stress, but low vigor seeds are affected severely, and lose viability. The test has subsequently been evaluated as an indicator of seed vigor in a wide range of crop species such as soybean, Brassica spp., sunflower, beans, clovers/alfalfa, maize, wheat, grasses etc and has been successfully related to field emergence and stand establishment. This test is inexpensive, simple, useful for all species and no extra know how is desired for correct evaluation, but the results may depend on moisture contents (McDonald, 1977) and differences between seeds in the rate of water absorption from humid atmospheres (Ellis and Roberts, 1980; Matthews et al., 1980).

Nascimento et al., (2007) studied accelerated aging test on pea seeds and recommended that the accelerated aging test was efficient for vigor evaluation. The most adequate condition for evaluating vigor involved 41 C, a period of 48 hrs and the presence of saturated NaCl solution.

### **2.5.3 Biochemical Tests**

#### **2.5.3.1 Electrical conductivity test**

The conductivity test is a measurement of electrolytes leaking from seeds. This test was first introduced by Fic and Hibbard in 1925 (Copeland and McDonald, 1995). It was later developed into a routine vigor test to predict field emergence of garden pea and used by various workers. (Matthews and Bradnock 1968; Bradnock and Matthews, 1970; Carver and Matthews, 1975; Scott and Close, 1976; Hampton and Scott, 1982; AOSA, 1983; Hadavizadeh and George, 1989; Parera et al., 1995; ISTA, 1995). The test measures the cell membrane integrity and is based on the premise that low vigor (deteriorated) seeds have poor membrane integrity. As seed rehydrates

during early imbibition, the ability of its cellular membranes to reorganize and repair any damage that may have occurred will influence the amount of electrolyte leakage from seeds. The greater the speed with which the seeds are able to re-establish their membrane integrity the lower the electrolyte leakage. Higher vigor seeds are able to reorganize their membranes more rapidly, and repair any damages to a greater extent, than lower vigor seeds. Consequently, electrolyte leakages measured from high vigor seeds are less than that measured from low vigor seeds.

Machado et al., (2011) studied the suitability of electrical conductivity test in pea seeds and concluded that the electrical conductivity test is sensitive to differentiate seed lots of forage pea whenever conducted with 250 ml of water under 25°C for 24 hours. Hence it plays an important role in seed vigor testing.

According to Processors and Growers Research Organization (P.G.R.O., 1981), the conductivity values are translated into vigor grades as follows:

24 $\mu$ s or less	High vigor	Suitable for sowing in early drillings
25-29 $\mu$ s	Medium vigor	Suitable for early sowing depending on the seed bed conditions
30-43 $\mu$ s	Low vigor	Not suitable for early sowing
Above 43 $\mu$ s	Very low vigor	Not suitable for sowing at all

The electrical conductivity test is a rapid, cheap and simple test. Several factors affect the test results such as, pH, soaking temperature, soaking duration, temperature at evaluation, initial seed moisture contents and seed size (Matthews and Bradnock, 1968; Bradnock and Matthews, 1970; Tao, 1978).

The conductivity test had been successfully used and recommended for crops such as, corn and soybean (AOSA, 1983; Barros and Marcos 1997), barley (Shephard et al., 1996), rice (Krishnasamy and Seshu, 1990), cabbage (Taylor et al., 1995) and bean (Fernandez and Johnston, 1995).

Wang et al., (2003) studied vigor tests and field emergence for several forage species in China. Their results showed that compared to standard germination (SG), electrical conductivity (EC) and controlled deterioration (CD) tests provided better parameters in assessing seedlot quality of *Vicia benghalensis* and predicting field emergence for the *Vicia benghalensis* and *Medicago sativa*.

### **2.5.3.2 Tetrazolium test**

The tetrazolium test is one usually used to test seed lot viability, and can also be very useful in determining seed vigor, (ISTA, 1995). The test evaluates the presence and location of living tissue within the seeds through the reaction between a solution of 2, 3, 5-triphenyl tetrazolium chloride (TZ) with active dehydrogenase enzymes. The TZ is reduced by enzymes to produce the red and stable substance called as formazan. Hence, the presence of red staining indicates living tissue; dead tissue remains unstained. The viable seeds are evaluated more critically into categories of high, medium and low vigor. a) Staining is uniform and even, tissue is firm and bright. (high vigor) b). Embryo completely stained or embryonic axis stained in dicots and extremities may be unstained, some over stained/less firm areas exist, (medium vigor) c). Large areas of non-essential structures unstained; Only one root stained (monocots) or extreme tip of radicle unstained (dicots) and the tissue are milky, flaccid and over stained, (low vigor).

A major benefit of the test is a very rapid estimate of the vigor of viable seeds. This test has been used extensively on cereal crops and results for field pea have shown good relationships with field performance.

This test has also been used and recommended in other crops such as maize, soybean, cotton, and clover and other large seeded legumes. (AOSA, 1983). Rech et al., (1999) recommended that tetrazolium test was efficient for rapid evaluation of seed physiological quality in pea.

Rech et al., (1999) studied the quick evaluation of the physiological quality of pea seeds and concluded that pH test for individual seed exudates with 30 minutes of imbibition rapidly evaluated seed viability; tetrazolium and conductivity tests were efficient for rapid evaluation of seed physiological quality, whereas pH test of the bulk seed exudates, correlates with seed vigor. Barros et al., (2005) concluded that the tetrazolium test was efficient in evaluating squash seed viability and seed vigor.

### **2.5.3.3 Respiration activity**

Seed germination and seedling growth require the use of metabolic energy acquired from respiration. This test is based on the assumption that seeds showing high respiration rate can produce vigorous seedlings (AOSA, 1983). Woodstock and Pollock (1965) quoted in AOSA (1983) reported that during the first few hours of imbibition, respiration was closely related with seedling growth rate in beans.

Woodstock (1969) quoted in AOSA (1983) grouped corn seeds into high, medium and low vigor ones by using the differences observed in respiration rates.

Similar correlations between respiration rates and seedling growth rates have been found in wheat and soybean.

#### **2.5.3.4 Glutamic acid decarboxylase activity (GADA)**

Glutamic acid decarboxylase (GADA) is an enzyme which was first observed to be activated in wheat seeds during imbibition and later it was recognized also as a better indicator of storability of seeds than the level of free fatty acids. Determination of this enzyme is known as GADA test.

The procedure for measuring GADA is simple. The equipment consists of water bath or temperature control, manometers, a scale for measuring manometer fluid moments, ½ pint jars and a small grinder. Seeds are grinded and placed in the jar with buffered glutamic acid (Bedell, 2001). The glutamic acid decarboxylase (GADA) reacts with glutamic acid and glutamate is converted into aminobutyric acid which results in released of CO<sub>2</sub> (Hampton, 1994). The amount of CO<sub>2</sub> evolved reflects the level of enzymatic activity. Measurements are usually taken after 30 minutes at 26°C (Bedell, 2001). High vigor seeds usually produced more amount of CO<sub>2</sub> and vice versa in low vigor seeds. Grabe (1964) quoted in AOSA (1983) worked a lot in developing the GADA test to one of the potential values in vigor testing. He found that GADA was highly correlated with seedling growth in corn.

### **2.6. CORRELATION BETWEEN FIELD EMERGENCE AND LABORATORY TESTS**

Most definitions of seed vigor emphasize the relationship between seed vigor test and field emergence and many studies have demonstrated that this association exists. Consequently, the ultimate value of any seed vigor test would be its ability to predict field emergence. Field emergence depends on interaction between the seed vigor and seed bed environment.

Jones and Peterson (1976) used seedling height as primary criteria for vigor evaluation in rice and proposed that performance in laboratory was directly correlated with field emergence. Yaklich and Kulik (1979) performed laboratory test for standard germination and seedling vigor classification in 144 seed lots of soybean. Most of the laboratory test measurements were significantly correlated with field performance.

Balbaki and Copeland (1987) correlated several vigor tests with field data in winter wheat and reported that cold test and accelerated ageing tests were good indicators of field performance and storage capacity. Venter van de et al. (1993)

observed in wheat seeds that percent germination at low temperature (9°C) had higher correlation with percent field emergence than other seed vigor tests like accelerated aging test and complex stressing vigor test.

Ajala, (1999) studied four cultivars each of pigeon pea and winged bean for various laboratory and vigor tests and compared the results with field emergence. He found that seedling vigor and electrical conductivity measurements were the most sensitive indicators in the prediction of field emergence of winged bean and pigeon pea. As per Ilbi et al. (2009), cool germination test at 18°C could be used as alternative test for seed vigor test in corn as it provided high correlation with field emergence and with cold test.

Fernandez and Johnston (1995) performed bulk conductivity test in lentil, bean and chickpea and found that bulk conductivity test correlated well with field emergence. Makkaw et al., (1999) {B} studied relationship between seed vigor tests and field emergence in lentil (*Lens culinaris* Medikus) in Sudan and Syria, and concluded that speed of germination, seedling dry weight and electrical conductivity had the best correlation with field emergence in both clay and silty loam soils. Miguel and Cicero (1999) (B) conducted a study to assess the efficiency of the cold test for soybean (*Glycine max*) seed vigor determination. They found that cold test using paper rolls without soil exhibited the best correlation with first germination count, electrical conductivity and field emergence.

Roosrokh et al., (2002) conducted a study to investigate the relationship between seed vigor and field performance in chickpea cultivars Jam and Kaka, grown at 2 locations in Tabriz, Iran, in 1997. They found that vigorous seeds had significantly better performance than deteriorated seeds. Correlation among traits showed that among the seed vigor tests, the electrical conductivity and germination speed were significantly correlated with seed yield.

Sridhar and Nagaraja (2004) studied the relationship between vigor tests and field emergence in maize, sorghum, cotton, pigeon pea and green gram. They concluded that the speed of germination, germination rate, and vigor index were positively correlated to field emergence, while electrical conductivity was negatively correlated to field emergence. Seedling dry weight had no significant association with field emergence either in fresh or in revalidated seeds of any crop.

Peksen et al., (2004) studied relationships among some seed traits, laboratory germination percentage and seedling emergence under field conditions in pea (*Pisum*

*sativum* L.) in Turkey. They found that 100-seed weight was negatively and highly significantly correlated with laboratory germination and field emergence percentages. However electrical conductivity showed positive and highly significant correlation with 100-seed weight, while it was negatively and highly significantly correlated with laboratory germination and field emergence percentages.

Amaritsut (2004) developed standard patterns of TZ for vigor test in soybean. He proposed that high vigor and medium vigor seeds according to TZ test would represent field emergence percentage. It was proven that high correlations were found between the TZ vigor test results and field emergence.

Maurya, et al., (2006) studied various seed vigor tests and their correlation with field emergence in oat (*Avena sativa* L.) and concluded that seedling dry weight, seedling length, seedling vigor index, standard germination, viability and first count showed significantly positive correlation with field emergence test.

Shukla et al., (2009) studied seed quality parameters and correlation analysis of seed yield and vigor of pea. They found that seed vigor parameters had strong and positive association with field emergence, while seedling root lipid content had highly positive correlation with germination percentage and seedling growth.

Bento et al., (2010) investigated efficiency of vigor tests for evaluation of physiological quality of *Erythrina velutina* wild seeds. They found that germination, first germination count, speed of seedling emergence and accelerated ageing tests coincided with seedling emergence test results in the greenhouse. Ishrat et al., (2012) (B) studied the correlation of vigor traits with field emergence in rice. They reported that paper piercing ability and cold tolerance of seedlings had a direct association with field emergence.

## **2.7 GENETIC AND ASSOCIATED STUDIES**

The knowledge of inheritance mechanism of seed and seedling traits is very important for a plant breeder to formulate appropriate breeding strategy for the improvement of any particular trait. Pollock and Roos (1972) divided seed and seedling vigor into two components, i.e. genetic and physiological and reported that genetic component included differences between two or more genetic lines and the physiological components included differences between seed lots within one genetic line.

Townsend (1974) reported the existence of highly significant differences among progenies of Cicer milk-vetch (*Astragalus cicer* L.) for speed of germination and seedling emergence. Variation for seedling vigor was observed and it was suggested that improvement in seedling vigor is possible through breeding.

The information related to various genetic aspects of current study is revived as under:

### **2.7.1 Variability, Heritability and Genetic Advance**

Heritability is one major component of response to selection for successful breeding programme. The selection capacity of the population mainly depends on the amount of heritable variation present. Heritability in the “broad sense” was defined by Lush (1940) as the ratio expressed in percent of genetic variance to total phenotypic variance. Heritability estimates have been found to be helpful in earlier selection on the basis of phenotypic performance of quantitative traits.

Sureja and Sharma (2000) studied genetic variability, heritability and genetic advance in a collection of 30 indigenous and exotic genotypes of garden pea. Considerable genetic variability for pod yield and its component characters were observed. High heritability in association with high genetic advance observed for plant height, length of internode, pod yield/plant, number of pods/plant, seed yield/plant, number of primary branches and 100-seed weight, indicating additive gene effects and emphasized the effectiveness of selection for these traits to improve economic yield.

Chaudhary and Sharma (2003) studied genetic variation, correlation and path analyses for yield and yield components in pea. They found significant genetic variation among the F1 hybrids for all the characters. Plant height, number of pods per plant and first flowering node recorded the greatest phenotypic coefficient of variation. The estimates of heritability were highest for plant height. Plant height and pod yield exhibited the greatest genetic gain. High heritability coupled with high genetic advance was observed for pod yield per plant, plant height, number of pods per plant and 1000-seed weight.

Kaloo et al., (2005) studied forty-seven genotypes of vegetable pea, which were selected from four hundred twenty three lines based on the plant growth, pod and seed morphological characteristics. Significant variability for all the traits was observed with the maximum variation for plant height and yield per plant. First flowering node had positive significant correlation with days to flower and plant

height. The pod length and average pod weight had significant and positive correlation.

Sirohi et al., (2006) conducted an experiment on 25 genotypes of pea and found that plant height had highest estimates of phenotypic as well as genotypic coefficients of variation. The estimates of PCV were higher than the estimates of GCV for all the thirteen characters studied. Among the inter se associations days to flowering with days to maturity , number of pods per plant , pod length and number of primary branches with plant height number of pods per plant , pod length and plant height with pod length harvest index and plant height with number of pods per plant showed positive and significant associations at phenotypic level. The number of seeds per pod exhibited highest direct effects towards seed yield at genotypic level.

Gupta et al., (2006) studied genetic variability and heritability in garden pea (*Pisum sativum* L.). The analysis of variance revealed highly significant differences for all characters studied. Both phenotypic and genotypic coefficients of variation were highest for green pod yield. Heritability ranged from 36.05% for total soluble solids to 99.16% for early yield per plant. Genetic advance was highest for green pod yield per plant. High heritability coupled with high genetic advance was observed for days to first flowering nodes, plant height, number of first flowering nodes, dry matter weight per plant, green pod yield per plant and number of primary branches per plant, indicating the preponderance of additive gene effects.

Singh and Singh (2006) studied thirty-one advanced lines and 6 cultivars (control) of pea for assessment of genetic variability, heritability, genetic advance and character association for seed yield per plant and related attributes. They observed greatest variability for seed yield per plant, followed by number of pods per plant, plant height, number of branches per plant, and 100-seed weight. Estimates of heritability in the broad sense were high for all characters except number of days to flowering and pod length. High expected genetic advance coupled with high heritability estimates were predicted for seed yield per plant, number of pods per plant, and plant height, indicating the low variation due to the environment. Seed yield per plant had significant and positive association with number of pods per plant, plant height, harvest index, and number of grains per pod.

Salam et al., (2007) conducted experiments to analyze the genetic architecture of yield and its components in different field pea genotypes. They concluded that the mean sum of squares due to the genotypes were significant for all the characters. High

variation was observed for most of the characters studied except for number of seeds per pod. High heritability coupled with high genetic advance was observed for plant height, height of first podding node, number of podding nodes, clusters per plant, seed yield per plant, branches per plant and pods per plant. Sardana et al., (2007) assessed genetic variability in 210 accessions of pea germplasm assembled from diverse eco-geographic regions of the world. They found moderate to high levels of genotypic coefficient of variability, heritability, and genetic advance for plant height, 100-seed weight, pod length, seed yield/plant, and pods/plant which suggested that these traits would respond to selection owing to their high genetic variability and transmissibility.

Nawab et al., (2008) studied twelve genotypes of pea to study the genetic variability, correlation and path coefficient of different traits. Analysis of variance revealed highly significant differences among genotypes for days to emergence, days to 50 percent flowering, number of pods per plant, weight of pods per plant (g), pod length (cm), number of seeds per pod, 100-seed weight (g) and green pod yield (kg/plot). Green pod yield per plot, 100-seed weight, number of pods per plant and weight of pods per plant showed high genetic coefficient of variation (GCV) along with high heritability and genetic advance, indicating good scope for selection.

Ceyhan et al., (2008) conducted Line x tester analysis in pea (*Pisum sativum* L.) for the identification of superior parents for seed yield and its components. The results depicted that broad genetic variability among the genotypes. Estimates of variance due to GCA and SCA and their ratio revealed predominantly non-additive gene effects for all studied traits. Hybrids generally showed greater yield potential than their parental genotypes. Heterosis was observed in some hybrids for each trait studied.

Jitendra et al., (2010) studied variability and character association in garden pea. They found high amount of genetic variability expressed by number of pods per plant, followed by yield per plot. It was moderate for duration of availability of green pods. Low phenotypic coefficient of variation was showed by 100-green seed weight followed by days to 50% flowering and days to first picking. Higher genetic advance was exhibited by number of pods per plant with their high magnitude of genotypic coefficient of variability and heritability indicating the presence of additive effects for these characters. Seed yield per plant exhibited significant and positive correlation with number of pods per plant, number of branches per plant and 100 green seed weight.

Lal et al., (2011) studied genetic variability and interrelation between yield and its contributing components in field pea. They revealed that the differences among 30 genotypes were significant for all the characters. Moderate to high levels of genotypic coefficient of variability (GCV), heritability and genetic advance were observed for plant height, number of primary branches per plant, pods per plant, seeds per pod, cluster per plant, 100 seed weight, harvest index, biological yield per plant and grain yield per plant. Among all traits plant height exhibited high estimates of GCV and PCV followed by biological yield and harvest index. Broad sense heritability was high for plant height and biological yield followed by number of pods per plant and cluster per plant, which suggested that these traits would respond to selection owing to their high genetic variability and transmissibility.

### **2.7.2 Line x Tester Studies**

Line x tester cross is a modified over the top cross scheme for inbred evaluation. The line x tester technique was developed by Kempthorne in 1957. It is a good approach for screening the germplasm on the basis of GCA and SCA variances and effects. The total number of crosses to be made is equal to the product of the number of lines and the number of testers included in the study. The line x tester technique has been extensively used in almost all the major field crops to estimate GCA and SCA variances and effects and to understand the nature of gene action involved in the expression of various quantitative traits. This technique measures the GCA and SCA variances and effects and the genetic components of variance.

#### **2.7.2.1 Heterosis**

“Heterosis” is defined quantitatively as an upward deviation of the mid-parent, based on the mean values of the two parents (Johnson and Hutchinson, 1993). Heterosis is manifested as improved performance for F1 hybrids generated by crossing two inbred parents. Heterosis may be positive or negative. Depending upon the breeding objectives, both positive and negative heterosis is useful for crop improvement. In general, positive heterosis is desired for yield and negative heterosis for maturity. Heterosis is expressed in three ways, depending on the criteria used to compare the performance of a hybrid. The three ways are: mid-parent, standard variety and better parent heterosis. However, from the plant breeders’ viewpoint, better parent (heterobeltiosis, Fanseco and Peterson, 1968) and/or standard variety (standard heterosis) are more effective.

Harinder et al., (2005) studied heterosis and combining ability for economic traits in genetically diverse lines of garden pea (*Pisum sativum* L.). They found that both general (gca) and specific combining ability (sca) variances were highly significant for all characters, which indicated that both additive and non-additive gene effects and are important for the inheritance of different characters studied. A large number of hybrids exhibited significant sca effects for yield and yield related characters. The best heterotic combinations were NDVP-9 x JP179, VP7906 x Arkel, Mithi Phali x MA-6, NDVP-8 x JP 179 and Mithi Phali x JP 179 for green pod yield, number of branches, number of pods per plant, plant height and days to marketable maturity, respectively.

Singh and Yadav (2005) studied heterosis and combining ability for economic traits from forty-five F1 hybrids produced in a line x tester fashion involving 18 parents (15 lines and 3 testers) for 11 characters (days to flowering, plant height, number of branches, number of pods per plant, pod length, number of seeds per pod, shelling percentage, 100-seed weight, days to marketable maturity, green pod yield per plant and dry matter content). They found that both general (gca) and specific combining ability (sca) variances were highly significant for all characters, which indicated that both additive and non-additive gene effects. Expression of heterobeltiosis was high for most of the characters except for days to flowering. The best heterotic combinations were NDVP-9 x JP179, VP7906 x Arkel, Mithi Phali x MA-6, NDVP-8 x JP 179 and Mithi Phali x JP 179 for green pod yield, number of branches, number of pods per plant, plant height and days to marketable maturity, respectively.

Raju and Muthiah (2007) undertook a study for twelve crosses alongwith their parents, viz., four lines (CO 5, CO 6, VRG 17 and LRG 41) and three testers (APK 1, ICPL 87119 and ICPL 332), of the line x tester mating design to study the combining ability and heterosis for seed yield and its components. A high degree of heterosis for seed yield per plant and other yield components over standard check (CO-5) was observed. The hybrid combination CO 5 x ICPL 87119 was considered as the best for higher seed weight and CO 6 x ICPL 87119 and CO 6 x ICPL 332 exhibited high heterosis for yield and other yield contributing characters.

Ganesh et al., (2008) crossed 15 pure lines of pea with three testers viz., HUP 9 (L1), Pusa 10 (L2) and F1 of HUP 9 x Pusa 10 (L3) to produce 3n families. Significant average heterosis over better parent was evidenced for plant height, pods

per plant and seed yield per plant. Heterosis for seeds per pod and seed weight was negative or low. The generation mean analysis revealed that the observed heterosis for seed yield per plant and pods per plant was mainly due to over dominance. Further, overdominance and higher magnitude of epistatic components (h) and (l) as was observed in modified triple test cross analysis, could be the possible cause of heterosis for seed yield per plant and pods per plant.

Jyothula and Guttala (2009) conducted heterosis and combining ability analysis for seed yield and some quantitative characters in field pea (*Pisum sativum* L.). The analysis of variance for combining ability revealed that both general combining ability (GCA) and specific combining ability (SCA) variances were highly significant for all the characters studied, which indicated that both additive and non-additive gene effects were important for the inheritance of different characters studied. The SCA variances were predominant compared to the GCA variances for all the characters indicating greater contribution of non-additive gene action in the expression of these characters. The best heterotic combination for number of clusters per plant, number of pods per plant, pod length and seed yield per plant was HUDP-15 x IARI-2899.

Kumar et al., (2009) studied combining ability and heterosis in pigeon pea (*Cajanus cajan* L.). Estimates of variance due to gca and sca indicated predominance of non-additive gene action for most of the characters. The cross combinations viz., LRG 30 x ICP 8863, PRG 100 x ICP 8863, LRG 30 x ICP 87119, ICPL 85063 x ICP 87119 and PRG 100 x ICP 87119 exhibited significant sca effects coupled with appreciable amounts of relative heterosis, heterobeltiosis and standard heterosis for yield and its attributes.

Borah, H. K. (2009) studied combining ability and heterosis in field pea (*Pisum sativum* L.). Heterosis was measured over standard check (SC) for ten characters in 15 hybrids. Considering the values of standard check, high heterotic effects were observed for days to maturity, protein content and 100 seed weight; moderate for days to flowering, number of seeds per pod, plant height and number of branches per plant. High heterotic response for seed yield per plant was mainly due to pods per plant, number of seeds per pod and 100 seed weight.

#### **2.7.2.2 Combining Ability**

Allard (1960) defined general combining ability as the average performance of a strain in a series of crosses and specific combining ability as the deviation from the

performance predicted on the basis of general combining ability. Combining ability initially was a general concept considered collectively for classifying an inbred line relative to its cross performance. Sprague and Tatum (1942) refined the concept of combining ability and the two expressions of GCA and SCA have a significant impact on inbred line evaluation and population movement. They defined GCA as the average performance of a line in hybrid combination and SCA as those instances in which certain hybrid combinations are better or poorer than would be expected on the average performance of the parent inbred lines included. They also emphasized that estimates of GCA and SCA are relative to and dependent on the particular set of inbred lines included in the hybrids under test, an important principle that is often forgotten.

Application of various biometrical techniques to understand the genetic architecture of seed vigor and its contributing components have been reported for a variety of crop plants. The combining ability analysis gives useful information regarding the selection of parents in terms of the performance of their hybrids.

Jahagirdar, J. E. (2003) conducted line x tester analysis for combining ability in pigeon pea. The combining ability analysis of 24 F1's of pigeonpea obtained from crosses between 3 lines and 8 testers along with their parents revealed significant non-additive gene action for almost all morpho-physiological traits, as variances due to specific combining ability (sca) were higher than the general combining ability (gca) variances. The parents BDN 2, ICPL 87, BSMR 736 and ICPL 87119 were the best general combiners for grain yield/plant, number of pods/plant, total biomass/plant and grain productivity/day. Ten out of 24 F1's showed significant positive sca effects and high per se performance for grain yields.

Pandey N. (2004) conducted line x tester crosses for the evaluation of general and specific combining abilities, variance components and standard heterosis for grain yield and yield components. Lines, DA 32, DA 34, DA 37, DA 46, DA 93-4, DA 93-2 and DA 94-6; Bahar (control); and testers DAMS-1 and ICPMS 3783 were good general combiners for seed yield per plant, and number of secondary branches, clusters and pods per plant. DAMS-1 was also a good general combiner for number of primary branches per plant and percentage of pod set. The partial dominance of additive genetic action was significant for number of pods per plant. The testers had greater contribution to the performance of the crosses.

Baskaran and Muthiah (2007) studied combining ability in pigeonpea [*Cajanus cajan* (L.) Millsp.]. They concluded the preponderance of non-additive gene action over additive for all the characters studied. The parents, CO 5, VBN 1, and ICPL 83027 were found to be good general combiners for majority of characters. The hybrids, VBN 1 x ICPL 83027, ICPL 87 x ICPL 83024, CORG 9701 x APK 1, CORG 9904 x ICPL 83027 had good sca effects for most of the traits including seed yield.

Sarode et al., (2009) conducted field studies, to estimate the combining ability variances and effects and the nature and magnitude of heterosis in different pea crosses for yield and yield components from 8 lines and 4 testers were mated in a line x tester fashion. Their results showed that KPSD1, PG 3 and HUVP 1 were the best general combiners for seed yield per plant, LMR 20, JP Batri Brown and KPSD 1 for pods per plant and HUVP 1, PG 3 and HUDP 15 for seeds per pod. A good agreement between specific combining ability effect and per se performance was observed for most of the characters. A total of 19 crosses showed significantly positive heterosis over better parent for seed yield per plant. Ten crosses showed more than 50% heterosis over the better parent, the highest by LMR 20 x JP Batri Brown (196.49%) and HFP 4 x JP Batri Brown (169.78%) for seed yield per plant. ●●●

### **2.7.3 Correlation and Path coefficient studies**

Correlation, measured by a correlation coefficient is important in plant breeding because it measures the degree of association, genetic or non-genetic between two or more characters. If genetic association exists, selection for one trait will cause changes in other traits called the correlated response. The cause of correlation can be genetic and/or environmental. Genetic causes may be attributed to pleiotropism and/or linkage disequilibrium. When genes are not closely linked, linkage disequilibrium is not an important cause of correlation between characters in random mated populations. In such cases the existence of genetic correlations is mostly attributable to pleiotropy (Hallauer and Miranda, 1982).

Rooszrokh et al., (2002) conducted a study to investigate the relationship between seed vigor and field performance in chickpea cultivars Jam and Kaka, grown at 2 locations in Tabriz, Iran, in 1997. They used electrical conductivity of leaked material from seeds, percentage of viable seeds, percentage of normal seedlings, speed of germination, and average dry weight of seedlings as the methods of study. Percentage and speed of seedling emergence were measured in the field. During

growth season, percentage of green cover, time of flowering, time to maturity, harvest index, yield components and final yield were determined. They found that vigorous seeds had significantly better performance than deteriorated seeds. Correlation among traits showed that among the seed vigor tests, the electrical conductivity and germination speed were significantly correlated with seed yield.

Chaudhary and Sharma (2003) studied genetic variation, correlation and path analysis for yield and yield components in pea. They found that the genotypic correlation coefficients were higher than the phenotypic correlation coefficients. Pod yield per plant showed positive phenotypic correlation with pod length, number of grains per pod, number of pods per plant and shelling percentage. Path coefficient analysis revealed that the number of grains per pod, pod length, number of pods per plant, and 1000-seed weight had the greatest direct effect on pod yield per plant. The greatest negative direct effects on pod yield were exhibited by number of days to 50% flowering. The number of pods per plant and number of grains per pod appeared to be the most important selection indices for green pod yield.

Mohan et al., (2005) subjected thirty-nine advance generation lines of garden pea to correlation and path analysis at both phenotypic and genotypic levels. They found that seed yield per plant was positively correlated with number of pods per plant, number of seeds per pod, shelling percentage and number of days taken from sowing to marketable maturity. Path coefficient analysis revealed that number of pods per plant and shelling percentage had the maximum direct effect on green pod yield.

Gupta et al., (2007) conducted experiments to study the character association and selection parameters in garden pea. They found that the genotypic correlations were higher than corresponding phenotypic ones for most of the characters implying inherent relationship among them. Green pod yield per plant showed significant positive correlation with pod length, 100-green pod weight, 100-green seed weight, number of green pods per plant and dry seed yield per plant at genotypic and phenotypic levels except TSS at phenotypic level, suggesting selection for longer pods, more number and weight of green pods and seeds, high TSS and more dry seed yield per plant would be highly desirable and effective.

Makkawi et al., (2008) studied correlation and path coefficient analyses of laboratory tests as predictors of field emergence in Lentil (*Lens culinaris* M.) in Sudan and Syria. They found that in Syria, no significant correlation was observed between vigor tests and field emergence. In Sudan, path analyses showed that

seedling dry weight and 100-seed weight consistently reflected the highest positive direct contribution towards field emergence in the two types of soil whereas the highest negative direct effect was shown by seedling growth rate, followed by cold soil test. Path coefficient analyses indicated that seedling dry weight (6.443, 5.778) and hundred seed weight (5.267, 3.973) had a positive direct contribution towards lentil field emergence in both types of soil. Likewise, in Syria the highest positive direct contribution was exhibited by seedling growth rate, followed by speed of germination and standard germination towards field emergence. 100-seed weight, seedling dry weight and cold soil consistently reflected the highest positive indirect contributions via seedling growth rate towards field emergence.

Schuab et al., (2008) assessed the physiological quality of soybean seeds by the germination test under water stress and correlated the results of the methodology with other vigor tests such as, seedling emergence in the field, standard germination (first and final count), seedling emergence in sand seedbed, speed of emergence, speed of emergence-index, speed of emergence-coefficient, accelerated aging, modified cold, tetrazolium (vigor and viability), electrical conductivity, seedling vigor classification, seedling length, seedling dry biomass and germination test under water stress at the osmotic potentials of 0 (control), -0.05, -0.1, -0.2, -0.4 and -0.6 MPa, induced by the use of polyethylene glycol (PEG 6000). They concluded that the germination test under water stress (-0.2 MPa), (evaluated after eight days), exhibited differences on the physiological potential of seeds from different cultivars.

Parimal and Chakraborty (2009) studied the relationship between seed coat colour and seed deterioration in green gram (*Vigna radiata* L.) in different environmental conditions. From the correlation coefficient analysis of pooled data of three years, they revealed that the germination percentage had positive and significant correlation with vigor index but had negative significant correlation with hundred-seed weight and electrical conductivity. Seedling fresh weight showed negative non-significant correlation with electrical conductivity. Vigor index exhibited negative significant correlation in all the situations with seed protein content, hundred-seed weight and electrical conductivity. Path analysis of pooled data of three years and in three different environmental conditions indicated that the germination percentage had negative direct effects on electrical conductivity. The maximum direct effect was exhibited by root-shoot ratio followed by vigor index.

Devi et al., (2010) studied the correlation, path coefficient and genetic divergence of different traits among the strains of pea. They revealed that days to first flower and seed yield had the highest positive and significant correlation with seed yield, length of internodes, days to first green pod harvest, number of primary branches, length of pods, breadth of pods, number of seeds per pod, number of pods per plant, pod yield per plant.

Saumya et al., (2011) conducted an experiment to study the character association among different quantitative characters and their direct and indirect effects on seed yield in garden pea crosses. Analysis of variance showed significant differences among all crosses for all characters, indicating that there is ample scope for selection of promising crosses from present gene pool for yield improvement in garden pea. Plant height, pods per plant and primary branches per plant showed positive and significant correlation with seed yield at genotypic and phenotypic levels, suggesting, their potential use as selection indices for improvement of garden pea genotypes for higher yield. Further, plant height and pods per plant exhibited high positive direct effect on seed yield.

#### **2.7.4 Gene Action**

Seed vigor and its various physiological components are controlled by polygenic systems. Features of polygenic inheritance, additive and non-additive viz. dominance, over-dominance and epistasis, all are found to be operative in such characters. Moreover, these characters are considerably influenced by both micro- and macro-environments. Sawicki and Boros (2000) studied the inheritance of some traits related to plant productivity in pea crossed in a diallel fashion at Radzikow, Poland, and recorded the observations in terms of, plant height, number of fruiting nodes, number of pods per plant, number of seeds per plant, weight of seeds per plant, 1000-seed weight and seed protein content. They found that plant height and protein content were controlled by additive gene action. Slight over dominance was found for number of fruiting nodes, number of seeds per plant and seed weight per plant. Over dominance was observed for 1000-seed weight. Dominant genes resulted in increasing plant height, number of fruiting nodes and components of plant productivity, whereas recessive genes increased protein content.

Sachan et al., (2001) studied combining ability and inheritance of seeds per pod and 100-seed weight in 10 diverse genotypes of field pea. They revealed that the variances due to general (GCA) and specific combining ability (SCA) were highly

significant for both characters, indicating the importance of both additive and non-additive gene effects. However, the magnitude of GCA variance was higher than that of SCA variance for 100-seed weight in F1, indicating the prevalence of additive gene effects. Estimates of GCA effects revealed that the parents DDR 4 and DMR 11 were good general combiners for seeds per pod, while Blue pea, Rachna, HUP 2 and KPMR 157 were good general combiners for 100-seed weight. KPMR 171 and KPMR 65 showed desirable GCA effects for both characters.

Meena et al., (2004) studied the Inheritance of seed shape and vigor in chickpea. The results on seed shape inheritance indicated the digenic control of seed shape and seed vigor in chickpea.

Singh et al., (2006) investigated the gene effects of yield and its component traits, i.e. days to flower initiation, node at which first pod appears, number of pods per plant, pod length, number of grains per pod, shelling percentage, 100-seed weight, plant height and number of primary shoots, as well as quality traits, i.e. dry matter, crude protein, total sugars and alcohol insoluble matter, in 6 generations of 2 crosses, i.e. Cross-I (Arkel x Matar Ageta-6) and Cross-II (Matar Ageta-6 x Bonneville) of pea. All the 3 types of gene actions, i.e. additive, dominance and interaction components, played a role in the inheritance of these traits, except for node at which first pod appears but degree differed with crosses. Duplicate type of epistasis was also observed in most of the characters.

Jyothula and Guttala (2009) conducted combining ability studies in 10x10 diallel set (excluding reciprocals) in field pea. They found that that gca and sca variances were highly significant for combining ability. The sca variances were predominant in comparison to gca variances for all the characters. This indicated that there was greater contribution of non-additive gene action in expression of these characters. The parents KPMR-660, DMR-44, IPF-26 and KPMR-663 were good combiners for seed yield per plant, number of pods per plant, number of seeds per pod and plant height. The F1 cross combinations KPMR-615 x DMR-44, KPMR-615 x KPMR-660 and DMR-44 x IPF-26 had significant desirable sca effects for seed yield per plant.

Vinod et al., (2011) crossed three testers, LFP 326, HUDP 15 and their hybrid, LFP 326 x HUDP 15, to 20 lines of pea to estimate additive, dominance and epistatic components of genetic variance for eleven characters by modified triple test-cross analysis. The total epistasis and its 'j+' type component were highly significant for all

traits. The 'i' type epistasis was evident only for pods per plant and harvest-index. The additive (D) and dominance (H) components of genetic variance were highly significant for all the traits. All traits showed partial dominance except over-dominance for pods per plant and seed yield per plant. The non-significant directional element (F) for all traits indicated ambidirectional nature of dominance.