EFFECT OF PLANT GROWTH REGULATORS ON MANIPULATION OF SOURCE-SINK RELATIONSHIPS IN PIGEONPEA (*Cajanus cajan* L.)

Dissertation

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
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in
BOTANY
(Minor Subject: Biochemistry)

By

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CERTIFICATE I

This is to certify that the dissertation entitled, "**EFFECT OF PLANT GROWTH REGULATORS ON MANIPULATION OF SOURCE-SINK RELATIONSHIPS IN PIGEONPEA (*Cajanus cajan* L.)**" submitted for the degree of **Ph.D.**, in the subject of **Botany** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ms. Kanchan Pahwa (L-2009-BS-44-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE – II

This is to certify that the dissertation entitled, **“EFFECT OF PLANT GROWTH REGULATORS ON MANIPULATION OF SOURCE-SINK RELATIONSHIPS IN PIGEONPEA (*Cajanus cajan* L.)”** submitted by **Ms. Kanchan Pahwa (L-2009-BS-44-D)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Ph.D.**, in the subject of **Botany** (Minor Subject: **Biochemistry**) has been approved by the Student’s Advisory Committee after an oral examination on the same.

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ABSTRACT

Field experiments were conducted at Punjab Agricultural University, Ludhiana during *kharif* season in the years 2010-11 and 2011-12 respectively with an objective to evaluate various groups of genotypes (early, mid, late and check) on the basis of pattern of growth, partitioning of dry matter, flower retention, growth efficiency and yield and to study the effect of foliar application of ethrel (100 and 200 μg ml^{-1}) and cobalt chloride (10 and 15 μg ml^{-1}) applied at vegetative and flowering stages on seed yield. Various morpho-physiological, biochemical and yield characteristics were analyzed at different developmental stages. Study conducted with early, mid, late and check genotypes of pigeonpea revealed marked differences in partitioning of assimilates to vegetative and reproductive parts at different stages of growth cycle. The higher CGR, RGR, leaf area, LAI, photosynthesis, stomatal conductance, pod set percentage in mid-duration group resulted in high yield. Further, reproductive efficiency of four mid-duration genotypes (AL 1578, AL 1593, AL 1702 and AL 201) was chemically manipulated by foliar application of ethrel and cobalt chloride. The parameters determining reproductive efficiency and yield were significantly improved due to reduction in flower abscission. Ethrel (100 and 200 μg ml^{-1}) sprayed at vegetative stage and $CoCl_2$ (10 μg ml^{-1}) sprayed at flowering stage efficiently improved seed yield and other biochemical traits through improved flower retention, pod formation , seed setting percentage and seed weight. Genotype AL 1578 exhibited high yield in all treatments including control as compared to all the genotypes. The leaves of treated plants exhibited higher level of chlorophyll, which provided better conditions for higher production of photosynthates to be utilized for grain yield due to larger sink resulting from increased branching and number of pods. The treatments also affected the content of various biochemical constituents (total soluble sugars, total soluble proteins, total starch content, total free amino acids, chlorophyll content as well as the activity of various enzymes viz., nitrate reductase (NR), invertase and PEP carboxylase). Correlation analysis showed significant positive relationships between plant height, crop growth rate, leaf area, number of pods/plant, pod set percentage, seed weight/plant, 100-seed weight and harvest index. Further, path analysis revealed leaf area, specific leaf weight and number of pods/plant had significant positive direct influence on yield. Path analysis of various biochemical traits with seed yield revealed that activities of nitrate reductase, total soluble proteins and total starch content in leaves and invertase activity, total starch in seeds showed positive direct effect on seed yield. These traits should be used as target traits to improve seed yield. Protein profiling in mature seeds revealed much denser bands in treated plants as compare to control and also number of bands were higher in treated plants. A study of pedicel anatomy revealed an increase in area of conducting tissues through PGRs application. This increase could be responsible for enhanced translocation of assimilates to flowers, thereby helping in their retention.

Keywords: Pigeonpea, partitioning, abscission, nitrate reductase, yield, protein profiling, pedicel anatomy

Signature of the major advisor **Signature of the student**

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ਨਿਚੋੜ

ਸਾਲ 2010-11 ਅਤੇ 2011-12 ਵਿੱਚ ਅਰਹਰ ਦੇ ਤਿੰਨ ਸਮੂਹਾਂ (ਛੇਤੀ, ਵਿਚਲੀ ਅਤੇ ਦੇਰ ਨਾਲ ਪੱਕਣ ਵਾਲੀਆਂ ਨਸਲਾਂ) ਦਾ ਵਿਕਾਸ, ਫੁੱਲ ਧਾਰਨ ਸ਼ਕਤੀ, ਵਿਕਾਸ ਨਿਪੁੰਨਤਾ, ਸੁੱਕੇ ਪਦਾਰਥਾਂ ਦੀਆਂ ਵੰਡਾਂ ਅਤੇ ਉੱਪਜ ਦੇ ਅਧਾਰ ਤੇ ਮੁਲਾਂਕਣ ਕੀਤਾ ਗਿਆ। ਇਹਨਾਂ ਵਿੱਚੋਂ ਚੋਣਵੀਆਂ ਫਸਲਾਂ ਉਪਰ ਬਨਸਤਪਤਕ (Vegetative) ਅਤੇ ਫੁੱਲ ਪੈਣ (Flowering) ਦੇ ਪੜਾਅ ਤੇ ਈਥਰਲ (100 ਅਤੇ 200 $\mu g\ ml^{-1}$) ਅਤੇ ਕੋਬਾਲਟ ਕਲੋਰਾਈਡ (10 ਅਤੇ 15 $\mu g\ ml^{-1}$) ਦਾ ਛਿੜਕਾਅ ਬੀਜ ਝਾੜ ਦੇ ਵਾਧੇ ਦੇ ਉਦੇਸ਼ ਨਾਲ ਕੀਤਾ ਗਿਆ। ਕਈ ਤਰ੍ਹਾਂ ਦੀ ਆਕ੍ਰਿਤਕ, ਜੈਵਿਕ ਰਸਾਇਣਕ ਅਤੇ ਝਾੜ ਲੱਛਣ ਦਾ ਵਿਸ਼ਲੇਸ਼ਣ ਵੱਖਰੀਆਂ ਵਿਕਾਸ ਸਥਿਤੀਆਂ ਤੇ ਕੀਤਾ ਗਿਆ । ਚਾਰ ਵਿਚਲੀਆਂ ਪੱਕਣ ਵਾਲੀਆਂ ਵਧੀਆ ਨਸਲਾਂ ਦੀ ਪ੍ਰਜਨਨ ਸ਼ਕਤੀ ਨੂੰ ਈਅਰਤਲ ਅਤੇ ਕੋਬਾਲਟ ਕਲੋਰਾਇਡ ਦੇ ਛਿੜਕਾਅ ਨਾਲ ਸੁਧਾਰਿਆ ਗਿਆ । ਛੇਤੀ, ਵਿੱਚਲੀ ਅਤੇ ਦੇਰ ਨਾਲ ਪੱਕਣ ਵਾਲੀਆਂ ਨਸਲਾਂ ਨੇ ਵੈਜੀਟੇਟਿਵ ਅਤੇ ਰੀਪਰੋਡਕਟਿਵ ਹਿੱਸਿਆਂ ਵਿੱਚ ਸੁੱਕੇ ਪਦਾਰਥਾਂ ਦੀ ਵੰਡ ਵਿੱਚ ਵੇਖਣ ਯੋਗ ਭਿੰਨਤਾ ਦਰਸਾਈ । ਜਿਆਦਾ ਸੀ ਜੀ ਆਰ, ਆਰ ਜੀ ਆਰ, ਪੱਤਾ ਖੇਤਰ, ਪ੍ਰਕਾਸ਼ ਸੰਸਲੇਸ਼ਨ, ਸਟੋਮੈਟਲ ਕਨਡੱਕਟੈਂਸ, ਫਲੀ ਲੱਗਣ ਦੀ ਪ੍ਰਤੀਸ਼ਤ ਦੇ ਕਾਰਨ ਵਿਚਲੀ ਪੱਕਣ ਵਾਲੇ ਇਕੱਠ ਨੇ ਵੱਧ ਝਾੜ ਦਿੱਤਾ । ਚਾਰ ਵਿੱਚਲੀ ਪੱਕਣ ਵਾਲੀਆਂ ਨਸਲਾਂ (ਏ ਐਲ - 1578, ਏ ਐਲ - 1593, ਏ ਐਲ - 1702, ਏ ਐਲ - 201) ਉੱਤੇ ਕੋਬਾਲਟ ਕਲੋਰਾਈਡ ਅਤੇ ਈਥਰਲ ਦਾ ਇਸਤੇਮਾਲ ਕੀਤਾ ਗਿਆ । ਪ੍ਰਜਨਨ ਸ਼ਕਤੀ ਅਤੇ ਝਾੜ ਨਿਰਧਾਰਤ ਕਰਨ ਵਾਲੇ ਮਾਣਕਾਂ ਦਾ ਵਾਧਾ ਘੱਟ ਫੁੱਲ ਝੜਨ ਕਾਰਨ ਹੋਇਆ । ਛਿੜਕਾਅ ਕੀਤੇ ਹੋਏ ਬੂਟਿਆਂ ਦੇ ਪੱਤਿਆਂ ਵਿੱਚ ਵੱਧ ਕਲੋਰੋਫਿਲ ਦੀ ਮਾਤਰਾ ਕਾਰਨ ਵੱਧ ਪ੍ਰਕਾਸ਼ ਸੰਸਲੇਸ਼ਨ ਦੀ ਕਿਰਿਆ ਹੋਈ ਜਿਹੜੀ ਕਿ ਵੱਧ ਝਾੜ ਪੈਦਾ ਕਰਨ ਵਿੱਚ ਮੱਦਦਗਾਰ ਸਿੱਧ ਹੋਏ । ਪ੍ਰਯੋਗਾਂ ਨੇ ਕਈ ਰਸਾਇਣਿਕ ਤੱਤ (ਪੂਰਨ ਘੁਲਣ ਵਾਲੀ ਖੰਡ, ਪੂਰਨ ਘੁਲਣ ਵਾਲੀ ਪ੍ਰੋਟੀਨ, ਪੂਰਨ ਸਟਾਰਚ ਤੱਤ, ਪੂਰਨ ਖੁੱਲ੍ਹੇ ਅਮੀਨੋ ਐਸਿਡ, ਕਲੋਰੋਫਿਲ ਤੱਤ, ਨਾਲ ਦੇ ਨਾਲ ਕਈ ਇੰਨਜਾਇਮਜ਼ ਦੀ ਕਿਰਿਆਵਾਂ ਜਿਵੇਂ ਨਾਈਟ੍ਰੇਟ ਰੈਡੂਕਟੈਸ, ਇਨਵਰਟੇਜ ਅਤੇ ਪੇਪ ਕਾਰਬੋਕਸੀਲੇਜ ਤੇ ਵੀ ਅਸਰ ਵੇਖਿਆ ਗਿਆ ਆਪਸੀ ਸਬੰਧ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਬੂਟੇ ਦੀ ਲੰਬਾਈ, ਫਸਲ ਉੱਪਜ ਦਰਜਾ, ਪੱਤਾ ਖੇਤਰ, ਫਲੀਆਂ ਦੀ ਗਿਣਤੀ, ਫਲੀ ਧਾਰਨ ਦੀ ਪ੍ਰਤੀਸ਼ਤ, ਬੀਜ ਪ੍ਰਤੀ ਬੂਟਾ ਭਾਰ, 100-ਬੀਜ ਭਾਰ ਅਤੇ ਝਾੜ ਵਿੱਚ ਸਾਕਾਰਾਤਮਕ ਸਬੰਧ ਦਰਸਾਏ ।ਪਾਥ ਕੋਫੀਸ਼ਿਏਂਟ ਮੁਲਾਂਕਣ ਨੇ ਪੱਤਾ ਖੇਤਰ, ਐਸ.ਐਲ.ਡਬਲਯੂ., ਭਾਰ ਅਤੇ ਝਾੜ ਦਾ ਸਿੱਧਾ ਸਬੰਧ ਦਰਸਾਇਆ । ਰਸਾਇਣਿਕ ਪ੍ਰਯੋਗਾਂ ਦੇ ਪਾਥ ਕੋਫੀਸ਼ਿਏਂਟ ਮੁਲਾਂਕਣ ਨੇ ਬੀਜ ਝਾੜ ਦਾ ਨਾਈਟ੍ਰੇਟ ਰੀਡੰਕਟੇਜ ਦੀ ਪ੍ਰਤੀਕਿਰਿਆ, ਪੂਰਨ ਘੁਲਣ ਵਾਲੀ ਪ੍ਰੋਟੀਨ ਅਤੇ ਪੱਤੇ ਵਿੱਚ ਪੂਰਨ ਸਟਾਰਚ ਤੱਤ ਅਤੇ ਇੰਨਵਰਟੇਜ ਪ੍ਰਤੀਕਿਰਿਆ, ਬੀਜਾਂ ਵਿੱਚ ਪੂਰਨ ਸਟਾਰਚ ਤੱਤ ਦੇ ਨਾਲ ਸਿੱਧਾ ਸਬੰਧ ਦਰਸਾਇਆ । ਇਹ ਲੱਛਣ ਬੀਜ ਝਾੜ ਦੇ ਵਾਧੇ ਲਈ ਟੀਚੇ ਦੇ ਤੌਰ ਤੇ ਇਸਤੇਮਾਲ ਕੀਤੇ ਜਾਣੇ ਚਾਹੀਦੇ ਹਨ । ਛਿੜਕਾਅ ਕੀਤੇ ਬੂਟਿਆਂ ਦੇ ਪੱਕੇ ਬੀਜਾਂ ਵਿੱਚ ਪ੍ਰੋਟੀਨ ਰੂਪ ਰੇਖਾ ਨੇ ਜਿਆਦਾ ਘਣੇ ਅਤੇ ਜਿਆਦਾ ਸੰਖਿਆ ਵਿੱਚ ਪ੍ਰੋਟੀਨ ਬੈਂਡ ਦਿੱਤੇ । ਫੁੱਲ ਦੀ ਡੰਡੀ ਦੀ ਘੋਖ ਨੇ PGRs ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਨਾਲ ਕੰਡਕਟਿੰਗ ਟਿਸ਼ੂ ਦੇ ਖੇਤਰ ਵਿੱਚ ਵਾਧਾ ਦਰਸਾਇਆ । ਇਸ ਵਾਧੇ ਕਾਰਨ ਫੁੱਲਾਂ ਨੂੰ ਵੱਧ ਅਸੀਮੀਲੇਟਸ (assimilates) ਮਿਲਦੇ ਹਨ ਜੋ ਕਿ ਇਹਨਾਂ ਦੀ ਧਾਰਨ ਸ਼ਕਤੀ ਵਿੱਚ ਮਦਦ ਕਰਦੇ ਹਨ ।

ਮੁੱਖ ਸ਼ਬਦ: ਅਰਹਰ ਦੀ ਦਾਲ, ਸੁੱਕੇ ਪਦਾਰਥਾਂ ਦੀ ਵੰਡ, ਨਾਈਟ੍ਰੇਟ ਰੀਡੈਕਟੇਜ਼, ਝਾੜ, ਪ੍ਰੋਟੀਨ, ਫੁੱਲ ਦੀ ਡੰਡੀ ਦੀ ਘੋਖ ।

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ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ

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ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

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CHAPTER I

INTRODUCTION

Pulses are basic ingredients in the diets of a vast majority of Indian population as they provide a perfect mix of high biological value when supplemented with cereals. Importance of pulses is relatively more in our country as its contribution in nutrient supply is far more than that in Asia and world as a whole. Each plant of a pulse crop is virtually a nature's mini nitrogen fertilizer factory, which enables it to meet its own requirement and also benefit the succeeding crops. In addition, pulses are excellent feed and fodder for livestock. Besides their dietary value and nitrogen fixing ability, pulses also play an important role in sustaining intensive agriculture by improving physical, chemical and biological properties of soil and are considered as excellent crops for diversification of cereal based cropping systems (Pothalkar 2007).

Pigeonpea (*Cajanus cajan L.)* is an important multi-use shrub legume of the tropics and subtropics. The crop originated in India and moved to Africa about 4,000 years ago. Pigeonpea belongs to the family Fabaceae sub-family papilionaceae. The genus '*Cajanus*' belongs to the sub tribe 'Cajanae' under tribe 'Phaseoleae' (Aiyer 1947). Duthie and Fuller (1883) found two species *Cajanus flavous* and *Cajanus bicolor* under the genus *Cajanus* and were called as 'tur' and 'arhar', respectively. The attempt of classification of pigeonpea was also made by Mahta and Dave (1931). Pigeonpea (2n=22) is a often pollinating and upto 30% out crossing has also been reported in this crop. This is probably a function of environment and populations of pollinating insects (Sheldrake 1984).

Pigeonpea (*Cajanus cajan* L.) is the fifth most important pulse crop in the world. Pigeonpea can be grown both as annual crop or perennial plants in homestead (Long and Lakela 1976, Liogier 1988). It is also known as red gram, congo pea, gungo pea, no eye pea, dhal, gandual and pois cajan. In India, it is second in area and 91% of world's pigeonpea is produced in India (Sodavadiya *et al* 2009). In year 2012, it was grown on 4.2 thousand hectares in Punjab and its total production was 3.9 thousand tonnes (Anonymous 2012). It also improves soil fertility through nitrogen fixation as well as from the leaf fall and recycling of the nutrients (Mapfumes 1993, Snapp *et al* 2002). It is an important pulse crop that performs well in poor soils and regions where moisture availability is unreliable or inadequate (Kimani 2001). It is the preferred pulse crop in dryland areas where it is intercropped or grown in mixed cropping systems with cereals or other short duration annuals (Joshi *et al* 2001).

Pigeonpea has a wide range of products, including the dried seed, pods and immature seeds used as green vegetables, leaves and stems used for fodder and the dry stems as fuel. Pigeonpea is consumed either as decorticated splits or green seeds as vegetables. It has been found that vegetable pigeonpea are considered superior to dry splits in crude fiber, fat, protein digestibility as well as trace elements and minerals. Besides its nutritional value, pigeonpea also possesses various medicinal properties due to the presence of a number of polyphenols and flavonoids. It is an integral part of traditional folk medicine in India, China, and some other nations. Pigeonpea is known to prevent and cure human ailments like bronchitis, coughs, pneumonia, respiratory. Dhal has 25% protein with a good balance of all amino acids except methionine and cysteine (Zu *et al* 2006 and Fu *et al* 2007).

The crop though accumulates maximum biomass, yet a proportionally low final yield is obtained due to higher partitioning of dry matter towards vegetative growth. Dry matter accumulation in the plant at progressive stages is a justified assessment of growth as a cumulative expression of different growth parameters. Further, it was observed that productivity of pigeon pea was not only dependent on accumulation of total amount of dry matter but its effective partitioning into economic sink seems to be key to increase the yield.

Excessive vegetative growth, indeterminate growth habit, lack of moisture stress tolerance, poor source- sink relationship, poor harvest index and poor biomass production in summer sown crop are some of the major physiological factors responsible for low productivity of pigeonpea (Chudasama and Thaker 2007).

Flower and fruit/pod drop is also one of the serious problems in this crop resulting in poor reproductive efficiency and consequently poor yield. Flower drop seems to be a wasteful strategy as it involves the loss of a considerable amount of energy and nutrients. However, the strategy appears primarily related to strong internal competition, in which a rapid purge of sink tissue may be useful (Doorn 2002). Flower abscission occurs both before and after fertilization. In some species, the mere lack of pollination, after a critical period, activates the abscission zones, and in other species the lack of fertilization, again after a critical period does so. Flower abscission in pigeonpea is known to be high (70-80%) without initiating pod setting percentage, but unlike other pulse crops weight of earlier and later pod set is same (Sheldrake and Narayanan 1979, Mallikarjuna and Saxena 2002, Sivaramakrishnan *et al* 2002). Therefore, the low yield in pigeonpea is due to poor pod set resulting from high flower and pod drops.

Its potential for high yield and wide adaptability can contribute to bridging the pulse shortage, if higher partitioning of dry matter is accomplished towards the storage sinks (Sharma *et al* 2009). Previous studies indicate the possibility of using pigeonpea to produce pulp for paper industry (Akhtaruzzaman *et al* 1986, Choudhury *et al* 2008). Pigeonpea is more feasible than any other possible pulses due to its special characteristics. It has high ability to be used as mixed crop and can be grown in unconventional lands like homesteads, roadsides, public places and borders of the crop fields (Saxena *et al* 2005, Kalaimangal *et al*

2008). However, it has been observed that unfertilized flowers often abscise due to competition for carbohydrates (Aufhammer *et al* 1987, Aloni *et al* 1996).

The movement of photoassimilates from the site of synthesis in leaf tissues (source) to the site of net accumulation in different tissues (sink) is under the potential control of numerous factors. Regulation of net flow of photoassimilates is an integrated process. The concentration gradient and ability of sink to assimilates between the source and sink is the primary determinant of the current rate of transport and pattern of partitioning. The intra and inter competition between flowers and immature pods associated with flower shedding, pod setting and pod filling is primarily determined by combination and balance of endogenous hormones. However, closer examination of various components involved in the overall process of partitioning indicates that endogenous plant hormones serve as modulators of many of the specific rate limiting constituents (Tekale *et al* 2009).

The physiological efficiency of a plant can be improved by prolonging photosynthesis, reducing photorespiration, better partitioning of photo assimilates, improving mineral ions uptake and stimulating nitrogen metabolism. The growth and development of plant is a complex process and is under the control of three main factors *viz*., genetics, environment and endogenous growth substances. The genetic factors determine the potentiality of a plant for growth and the fullest expression of this potential in turn is under the control of various environmental factors. The enhanced productivity of crop through physiological approaches is chiefly achieved by coordinating plant processes to synthesize maximum dry matter production and partitioning major quantum of this increased dry matter into effective yield contributing factors.

One of the reasons for new varieties giving increased yields is mainly because of the partitioning of larger proportion of their dry matter in economic parts of the plant. It may also be attributed to their better adaptation to the environment. These effects can also be achieved by certain growth regulators.

Plant growth regulators are substances which when added in small amounts modify the growth of plant usually by stimulating or inhibiting part of the natural growth regulation. They are considered as new generation of agrochemicals after fertilizers, pesticides and herbicides (Kumar 2001). Plant growth regulators like promoters, inhibitors or retardants play a key role in internal control mechanism of plant growth by interacting with key metabolic processes such as nucleic acid and protein synthesis.

Ethylene, a gaseous hormone has long been recognized as a growth inhibitor, but evidence is accumulating that ethylene can also promote growth (Pierik *et al* 2006). The stimulatory effect of ethylene is concentration and stage dependent. Ethylene is an inhibitor of cell division, cell expansion and transport of auxins which present expressive effects on the reduction of stem growth in length; however, it provides its radial expansion and horizontal orientation (Coll *et al* 2001). Ethylene released from ethrel (2-Chloroethylphosphonic acid) could possibly be utilized for promoting pod growth (Abbas 1991). Cobalt, an antagonist of ethylene is an essential element for the synthesis of vitamin B_{12}, which is required for human and animal nutrition. In higher plants cobalt is an essential element for legumes because of its use by micro-organisms in fixing atmospheric nitrogen. However, like ethrel its stimulatory effect is also concentration and stage dependent. Cobalt is required in low levels for maintaining high yields of tomato (Renner *et al* 2003), squash (Atta-Aly 1998), groundnut (Basu *et al* 2006), sweet potato (Gad and Kandil 2008) and potato (El- Bordiny and Gad 2008).

Exogenous applications of various hormones at different stages of fruit development and endogenous quantifications have highlighted their importance during fruit development (Chudasama and Thaker 2007). There is ample scope to improve source-sink relationship through PGR application to enhance the productivity.

Objectives

The present investigation was designed with the following objectives:

- Evaluation of early and late maturing pigeonpea genotypes for differential flowering behavior
- Identification of morpho- physiological and biochemical traits associated with yield
- To understand the physiological basis of flower retention and effective pod formation through the use of PGRs (ethrel and $CoCl_2$)
- To find out the correlation between various physiological and biochemical parameters with yield.

CHAPTER II

REVIEW OF LITERATURE

Pulses, besides being an indispensable component of vegetarian diet, play a vital role in sustaining long term productivity of soil through biological nitrogen fixation. The pulses will continue to be an important component of the crop production system. Among the pulses, pigeonpea (*Cajanus cajan* L.) is considered as one of the most important pulse crops of India, which accounts for 90 per cent of the world production, and has got high nutrition value. For the last decade, yield of common beans and other legumes have stagnated in comparison to a marked increase in cereal crops (Summerfield 1980).

In legume crops, many flowers are produced but only a few set pods are formed and result in low yield (Saitoh *et al* 2004, Mondal 2007, Islam *et al* 2010). The main reason for such low yields is abscission of flowers and immature pods. A major obstacle to increased yield of beans is premature and excessive abscission of flowers and young pods. Unfortunately the mechanism controlling abscission is not well understood.

The growth and development of a plant is a complex process and is under the control of three main factors *viz*., genetics, environment and endogenous growth substances. The genetic factors determine the potentiality of a plant for growth and the fullest expression of this potential in turn is under the control of various environmental factors. The enhanced productivity of crop through physiological approaches is chiefly achieved by coordinating plant processes to synthesize maximum dry matter production and partitioning major quantum of this increased dry matter into effective yield contributing factors.

Plant growth regulators (promoters, inhibitors or retardants) play key role in contributing internal mechanisms of plant growth by interacting with key metabolic processes such as, nucleic acid metabolism and protein synthesis. Growth retardants are known to reduce inter-nodal distance, thereby enhancing source-sink relationship and stimulate the translocation of photo-assimilates to the seeds (Luib *et al* 1987). These natural substances may be applied directly on the plants (leaves, fruits, seeds), providing alterations in the vital and structural processes, in order to increase the production, improve quality and facilitate harvest (Castro 2003).

Growth regulators exert their influence upon foliar application in a number of ways. These could enhance the absorption by the leaf at the site of application, increase the migration within the leaf and/or stimulate the transport out of leaf in acropetal and basipetal direction. Although, the naturally occurring growth regulators (hormone) normally control plant growth, modification of growth can be achieved by the exogenous application of growth regulators. The following literature review gives an overview of the problem of abscission of reproductive structures in pigeonpea and other legumes and identifies some of the factors associated with the shedding of these organs. The information pertaining to "Effect of plant growth regulators on manipulation of source-sink relationships in pigeonpea (*Cajanus cajan* L.)" has been reviewed under the following headings:

2.1 Vegetative growth
2.2 Photosynthesis and translocation
2.3 Regulation of reproductive growth
2.4 Dry matter
2.5 Growth analysis
2.6 Yield and its attributes
2.7 Biochemical studies
2.8 Anatomy of inflorescence

2.1 Vegetative Growth

The developing pods require considerable amount of assimilates for their growth and maintenance. The leaves which subtend the developing pods generally senesce at faster rate, while the branches/leaves without pods in their axil remain green. The relationship between the growing sink and source is of vital importance for determining the potential productivity of the plant (Gifford and Evan 1981).

Chettri (2003) observed that seed yield depends on LA, branch and nodule number plant^{-1} in soybean. Tandale and Ubale (2007) also observed that seed yield depends on LA and TDM production in soybean. Malek *et al* (2012) postulated that high yielding genotypes always showed superiority in TDM production and LAI as compared to low yielding ones at most of the growth stages. These results also indicate that LAI and TDM are the most important parameters for increasing seed yield in soybean.

Mehra *et al* (1987) observed that specific leaf weight was an important trait in the process of storage of photosynthates and their subsequent translocation to developing seeds. Specific leaf weight (SLW) has been reported to have a strong positive association with leaf photosynthesis in several crops (Bhatia *et al* 1996). A rapid crop growth rate has been found to be an important trait for selecting high grain yielding genotypes in cowpea (Marfo *et al* 1997) and in groundnut (Williams 1992).

The impact of foliar application of growth regulators at different concentrations on general growth parameters has been studied by many workers in various crops.

Bhattacharjee and Divakar (1989) verified the effect of ethylene @ 100, 500 and 1000 mg L^{-1} in *Jasminum* spp. and observed a decrease in the growth, increase in the number of branches and stem thickness and a late flowering period, although with a larger number of flowers. Tancredi (2004) removed the apical meristem of soybean plants at 25 cm height, and obtained an increase in number of branches and grain yield. In fact, the stress caused by pruning promoted an increase on the ethylene synthesis and also caused a break on the apical dominance, providing higher cytokinin content to the development of the lateral buds, which was responsible for the physiological responses.

Saxena *et al* (2007) proposed that high concentration of ethylene at post-flowering stage results in decline in dry matter production and leaf area, which retards growth of the plant. Further they suggested that the benefit of application of any plant growth regulator can be achieved if that is capable of increasing assimilate supply, which could inturn contribute to seed growth, since high ethylene levels at post-flowering stage do not contribute in this direction, this results in poor seed yield.

Campos *et al* (2010) proposed that although ethephon reduced plant height and first pod insertion, but plant achieved the height of approximately 75 cm and first pod insertion at 15 cm from the soil surface, and, moreover, provided higher branching, which can provide a larger yield of soybean.

Cobalt is an essential element for the synthesis of vitamin B12, which is required for human and animal nutrition (Young 1983). Jayakumar *et al* (2009) observed that cobalt at lower concentrations increased the yield of soybean by altering various physiological parameters.

Foliar application of Indole acetic acid (IAA) at three concentrations (12.5, 25, 50 ppm) induced increments of plant height, fresh and dry weights, number of branches and number of leaves per pod, weight of seeds per plant and weight of seeds per pod (El-Bassiouny and Shukry 2001, Sinsiri and Laohasiriwong 2007). El- Saeid *et al* (2010) reported that spraying cowpea plants with IAA caused significant effects on number of leaves and shoot dry weight. IAA @ 100 mg/L caused significant decrease in plant height. However, at concentrations of 25 and 50 mg/L, IAA application caused significant increase in number of leaves and shoot dry weight per plant. The same trend was seen during flowering and abscission time.

Pigeonpea has small leaves that are produced at a slow rate so the time to achieve full radiation interception is substantially delayed as compared to other species. Consequently, major increase in yield potential of pigeonpea is possible if the rate of leaf area could be increased (Muchow 1985). Leite *et al* (2003) observed that foliar application of GA_3 increased the plant height, first nodal elongation and stem diameter in soybean. Leaf area and dry matter production also increased; however, there was no effect on number of leaves, stem branches and root dry matter. Kothule *et al* (2003) reported that plant growth substances of different concentrations i.e. GA, NAA, CCC and salicylic acid each @ 100 and 200 ppm and urea @ 1 and 2% when applied exogenously as foliar spray improved morphological characters *viz*. plant height, number of branches, leaf area, total dry matter of plant and reduced the number of days to 50 per cent flowering in soybean.

Cato *et al* (2006) revealed that TIBA (30, 40 or 50 gm/l) application at V5 phenological stage in soybean (cv. Pintado) was effective in reducing plant height without affecting parameters related to productivity. Al- Desuqey *et al* (2007) reported that IAA, gibberellic acid or kinetin at different concentrations stimulated the growth vigor (root length, root fresh and dry weights and leaf area production) of cowpea throughout the growth periods. GA @ 200 ppm was found most effective in increasing plant height.

Emongor (2007) revealed that exogenous application of GA_3; 7 days after emergence at 30, 60 and 90 mg/L significantly increased plant height, first nodal height, leaf area and number of leaves per plant without significant effect on plant senescence in cowpea. Thus exogenous application of GA_3 can be used to modify growth and development of some cowpea varieties. Vasudevan *et al* (2008) reported that GA_3 (100 ppm) foliar spray at 50 per cent flowering resulted in production of significantly higher plant height, number of productive branches (6.68) and seed yield (8.53 q/ha) in fenugreek. Erulan *et al* (2009) reported that Seaweed liquid fertilizer (SLF) at low concentrations enhanced the growth parameters *viz*., shoot length, root length, leaf area, fresh weight, dry weight and moisture content.

Kim *et al* (1993) studied the effect of abscisic acid (50 ppm), benzyladenine (100 ppm), ethrel (1000 ppm), NAA (20 ppm) and TIBA (100 ppm) on soybean and observed that the growth regulators had no effect on time of maturity. TIBA treatment reduced plant height, particularly in the determinate cultivars. IAA at lower and moderate concentrations (25mg/L and 50mg/L) did not induce changes in stem length of cowpea plants, yet the higher level (100 mg/L) showed reduction in the same parameter (El-Mergawi 2003, El-Saeid *et al* 2010).

Sumabai *et al* (1987) observed that foliar spray of ascorbic acid, NAA and GA increased the vegetative growth and assimilate production and favoured longer reproductive growth in greengram. There was a significant increase in nitrogen content in leaves and stems of pea plants by spraying cytozyme, NAA and cycocel (Shende *et al* 1987). Similarly, Arora *et al* (1988) studied the effect of cytozyme (0.2%) and indicated that it promoted increase in height of potato plant. The maximum plant height in turnip was noticed with 150 ml/ha of cytozyme. While, the plant height was reduced when cytozyme concentration was increased to 200 ml/ha and foliar application of 100 ml/ha miraculan promoted the stem growth of turnip (Rana and Vashistha 1988).

Kumarvelu *et al* (2000) studied the effect of triacontanol (0.5 mg dm^{-2}) in greengram and indicated that it promoted the plant height when sprayed at 15 and 20 DAS. The foliar application of triacontanol (0.2 %) at vegetative, flowering and poding stages significantly increased the plant height, biomass production and better dry matter partitioning towards the sink in mungbean (Reddy *et al* 2002). Sinha *et al* (2002) found that ascorbic acid treated moong and pea plants showed an increase in the nodule number, nodule volume, dry weight of the plants and total nitrogen content as compared to control. While, Garai and Datta (2003) indicated that foliar application of cycocel reduced the plant height; increased the number of branches and nodules per plant in greengram. Prakash *et al* (2003) noticed that the foliar application of planofix (30 ppm) at 30 and 45 DAS and chamatkar (120 ppm) at 60 DAS recorded increased plant height, LAI and SLW in blackgram. Foliar spray of $ZnSO_4$ (0.5%) increased the plant height, number of leaves and branches per plant significantly over other treatments in pigeonpea (Varma *et al* 2004).

Ramarao (2004) noticed an increased pod yield in ICPL 85063 which might be due to higher plant height, RWC, SLA, number of pods per plant, test weight and HI, when evaluated the genotypes of pigeonpea for adaptation to drought under natural condition. The maximum grain yield was obtained with chelated iron (1 kg/ha) + N-P-K-S (20-18-17-20 kg/ha) and this treatment recorded 21.6 per cent increase in yield potential and there was improvement in plant height and number of branches per plant. Higher gross realization and net profit were recorded with the same treatment followed by $ZnSO_4$ (25 kg/ha) and sodium molybdate (2 kg/ha) in urdbean (Mevada *et al* 2005).

Patil *et al* (2005) revealed that spraying of NAA (25 ppm) resulted in significantly higher plant height, number of branches per plant as compared to control in mungbean. Foliar application of NAA (50 ppm) significantly increased the plant height, number of leaves and branches per plant compared to control in soybean (Thakare *et al* 2006). Similarly, Nehare *et al* (2006) reported that foliar application of NAA (20 ppm) significantly increased the plant height, test weight, biological yield as compared to control, triacontanol and ethephon in fenugreek.

2.2 Photosynthesis and translocation

Photosynthesis has generally been considered to be the primary factor affecting the dry matter production in crop plants. The dry matter production and its subsequent conversion into economic yield are the result of a complex physiological process within plants. Highest photosynthetic rate at flowering stage might be attributed due to higher leaf chlorophyll, higher stomatal and mesophyll conductance and lower intercellular CO_2 concentration. Photosynthesis showed strong correlation with leaf area and dry matter accumulation of blackgram (Biswas *et al* 2001). Manipulation of photosynthetic potentials has been practiced to increase crop productivity for a long time (Hossain *et al* 2009).

Bangerth (1989) developed two models supplementing each other to explain the abscission of generative organs. The competition model assumes that developing fruit and seeds, assimilates acceptors, compete with vegetative organs for resources. Under extreme circumstances insufficient supply of generative parts with nutrients can lead to abscission and less extreme circumstances leads to disturbed development of fruit and seeds both in terms of quality and quantity.

Cotton plants growing under favorable light conditions were shown to shed only 20% percent of their young fruits, but this increased to 90% when plants were placed in total darkness for 48 hr. The rise in abscission rate was correlated with greatly reduced translocation of carbohydrates into young fruits (Rabey and Bate 1978). Abscission of reproductive structures is also related to carbohydrate content. Horticulturalists have known for many years that plants with an abundance of carbohydrates are less likely to drop fruit than those with a low carbohydrate status. Retention of fruits by deciduous tree crops depends upon the accumulation of carbohydrate reserves in the previous season. Greater abscission of young fruits is observed when reserves are low (Addicott 1982). Not only TDM production, but also the capacity of efficient partitioning between the vegetative and reproductive parts may produce high economic yield (Shiraiwa *et al* 2004, Oh *et al* 2007).

Plant growth regulators are known to enhance the source-sink relationship and stimulate the translocation of photo-assimilates thereby helping in effective flower formation, fruit and seed development and ultimately enhance productivity of the crops. Growth regulators can improve the physiological efficiency including photosynthetic ability and can enhance the effective partitioning of accumulates from source and sink in the field crops (Solaimalai *et al* 2001).

The effect of cycocel in increasing chlorophyll contents may be due to the reduction in cell size resulting in dense cytoplasm (Appleby *et al* 1966). The increased chlorophyll content of Cycocel treated plants could be referred to hormonal effects as it stimulate chlorophyll biosynthesis through acceleration of chloroplasts differentiation and stimulating photosynthetic enzymes (Bekheta 2004, Bekheta *et al* 2006).

Saxena *et al* (2000) proposed that decrease in seed yield of chickpea by higher concentrations of ethrel could be due to enhanced leaf senescence leading to decrease in photosynthetic capacity of leaves and a decline in chlorophyll pigment as well as decreased supply of assimilates to developing fruit and seed. However, the low concentration of ethrel was found to be effective in increasing yield of that crop by improving source- sink relationships.

Use of growth regulators like MC, CCC and NAA is known to increase the rate of photosynthesis by increasing the chlorophyll content per unit area and the size of the mesophyll cells in the leaves. This leads to more rapid exchange of CO_2 into mesophyll cells by virtue of their larger surface area (Dulizhao and Oosterhius 2000, Kumar *et al* 2005).

Surendra *et al* (2006) reviewed that the treatment with GA_3 (25 and 50 ppm) gave significantly maximum LAI and SLW as compared to other treatments. Higher LAI is due to increase in leaf area which indicated higher photosynthetic surface area for prolonged period resulting in better yield. Further higher leaf area could be due to the role of auxins and gibberellins in cell division, cell elongation and reducing the abscission of leaves by regulatory senescence. The increase in SLW indicates increased leaf thickness due to stacking of palisade cells and moreover, okra being C_3 plant has low photosynthetic efficiency and such increased leaf thickness could probably enhance the photosynthetic efficiency due to stacking of mesophyll and bundle sheath cells there by recapturing the CO_2 released in photo respiratory process and leading to increase in dry matter production.

Tanaka and Fujita (1979) measured the sugar and starch content in bean pod walls at 20 days after bloom, they found that normally growing pods had much higher sugar and starch contents than those senecsing and devoid of seeds. A shortage of photosynthate during pod wall growth was proposed as one of the causes of abortion.

Supplying bean explants with sucrose considerably enhanced starch deposition and retarded abscission of leaflets (Addicott 1982). Setting and abscising legume pods differ in their carbohydrate content. Dybing *et al* (1986) reported that normally setting soybean pods accumulated higher levels of soluble carbohydrate and starch than distal pods which usually abscise. They suggested that pods at the base of the raceme can better compete for photosynthate than those at distal positions and, therefore, have higher carbohydrate levels. On the other hand, a depletion of stored reserves in distal pods would be expected to accompany a lower photosynthate supply.

Plant physiologists refer to translocation of major solutes in phloem as representing the source/sink relations of a plant, a feature that determines harvest index (ratio of grain yield to plant biomass at final harvest) and has been the single most important trait exploited in domestication of a species and its improvement by plant breeders. Despite the obvious significance of this relationship, the means by which it is regulated remain elusive, but intuitively some sort of signaling mechanism has been invoked. Trewavas (2006) points out that in whole plants ‘‘both sugars and N-solutes are obvious candidates to provide information loops enabling some balance between root and shoot development to be maintained. The essence of soybean yield formation is the process of interactions among source, sink and “flow” (Liu *et al* 2010).

Translocation or long distance transport in plants is achieved by a vascular network that connects and is an integral part of all organs. The vasculature comprises two distinctly different and separate cellular translocation pathways: xylem and phloem. The principal xylem pathway is the transpiration stream that moves nutrients and water taken up by roots to the shoot. This stream also bears products of root metabolism and solutes that reflect features of the internal and external root environment. Phloem provides the means for redistributing xylem-delivered solutes to weakly transpiring organs, but most significantly phloem distributes the carbon assimilated by photosynthesis (principally as Sucrose) to heterotrophic organs like roots, vegetative and reproductive apices, flowers, fruits, and developing seeds. Together, these two translocation streams provide all the nutrients and assimilates in appropriate forms and proportions to enable growth and development in an ordered and regulated fashion (Atkins and Smith 2007).

Translocation connects distant components of the plant body, xylem and phloem have long been considered to fulfill a role in communicating between organs, through the movement of plant hormones and other signaling molecules. Such signals are envisaged to move with assimilates by mass flow. However, phloem also transmits pressure/concentration (turgor) information at rates greatly in excess of mass flow of solutes (Thompson and Holbrook 2004) and long distance electrical signaling is also thought to be directionally propagated via vascular bundles (Brenner *et al* 2006).

Chandrasekhar and Bangarusamy (2003) reported that foliar application of growth regulating chemicals and nutrients in combination of 100 ppm salicylic acid, 2% DAP, 1% KCl and 40 ppm of NAA had given higher grain yield in greengram under irrigated conditions. The causes for the increase in yield are the increased dry matter production and efficient assimilate translocation to the developing sink leading to increased number of clusters and flowers per plant that ultimately resulted in higher grain yield.

Chudasama and Thakar (2007) proposed that fruit development requires a major investment of carbon and water. Photosynthate supply plays an important role in controlling crop reproductive development under well watered conditions. Pods are part of a source-sink pathway that can produce photosynthates and deliver nutrients to the seeds. Higher amount of GA in larger pods and seed may increase uptake of water in the whole fruit. They further proposed that endogenous GA and WC of seed and pod play an important role in increasing fruit size of pigeonpea.

2.3 Regulation of Reproductive growth

Flower and fruit/pod drop is one of the serious problem in pulses resulting in poor reproductive efficiency viz. the number of flowers formed/retained and the percent fruits developed with normal seeds and consequently poor yield (Saxena *et al* 2007). Abscission of fertilized flowers is often stimulated by stress, such as adverse growth conditions, lack of nutrients and by competition for assimilates (Stephenson 1981, Lee 1988, Aneja *et al* 1999). In most of the species, abscission zone is found in the pedicel, the stem segment that subtends the ovary. The zone usually occurs either towards the top or base of the pedicel (Doorn 2002).

Doorn (2002) postulated three reasons for assuming that fall is due to true abscission. 1. Flower fall also occurred in experiments in which flowers did not show any visible symptoms of senescence, 2. The separation zone was smooth and clear cut, 3. Abscission always occurred at the same place, about 0.5 cm above pedicel base. According to Egli (2009) much of the variation in yield of soya bean [*Glycine max* (L.) Merrill] and other grain crops is associated with changes in the number of pods and seeds per unit area. Photosynthesis and seed characteristics are the primary determinants of pod and seed number, but recent research suggests that the temporal distribution of flower production may also play an important role.

The chemical manipulation of plant growth and development is an approach, which has considerable potential for the quantitative as well as qualitative improvement of crop performance. Plant growth regulators (PGRs) which can improve the physiological efficiencies of plant could play a significant role in increasing crop yield and quality, when applied as foliar spray at proper crop growth stage in optimum concentration (Garai and Datta 2003). Sekhar *et al* (2001) suggested that there is vast scope for the use of growth regulators and nutrient sprays in pigeonpea for achieving proper nicking between diverse genotypes varying in flowering duration. The ethrel and MH and NAA can be used for proper synchronization of flowering in pigeonpea. Benzyladenine has been widely used in soybean to improve crop productivity by increasing pod set (Peterson *et al* 1990, Saxena *et al* 2003).

Salicylic acid ($C_7H_6O_3$) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plant, such as stomatal closure, ion uptake, transpiration and stress tolerance (Khan *et al* 2003 and Shakirove *et al* 2003). Foliar application of salicylic acid exerted a significant effect on plant growth metabolism when applied at physiological concentration and thus acted as one of the plant growth regulating substances (Kalarani *et al* 2002). Salicylic acid increased the number of flowers, pods/plant and seed yield of soybean (Gutierrez-Coronado *et al* 1998); enhanced maize growth (Sheheta *et al* 2001, Abdel-Wahed *et al* 2006, El-Mergawi and Abdel-Wahed 2007).

Ethylene released from ethrel (2-Chloroethylphosphonic acid) at 250 ppm promoted pistillate flower formation in cucurbits (Robinson *et al* 1970). Ethrel could possibly be utilized for promoting pod growth as Abbas (1991) has shown that early pod development is related to higher ethylene levels, thus decreasing flower and pod shedding and thereby reducing abscission and improving better pod set. Ethrel induced increase in cell division, resulting in increase fruit size and yield have been reported in tomato fruits (Atta – Aly *et al* 1999).

Cycocel (2-Chloroethyl, trimethyl ammonium chloride) has been used to check the abscission of flower and modify the crop canopy for improving the yield in gram (Bangal *et al* 1982), pigeonpea (Vikhi *et al* 1983) and soybean (Singh *et al* 1987). Grewal *et al* (1993) reported that cycocel improves the translocation of photosynthates. More protein content stored in the seeds might be due to improvement of translocation of photosynthates to the seeds. Sexton *et al* (2000) reported that ethylene had little effect on abscission in some vegetative tissues. However, abscission of generative tissues such as petals is generally sensitive to ethylene. Doorn (2001) found that petal wilting was either ethylene insensitive and this was also consistant within families or subfamilies. Flowers of plants growing in field usually fell after petal wilting, whereas following exposure to exogenous ethylene, the petals were turgid when the flowers were shed.

Shehata and Bondak (1996) found that there was relationship between increase of endogenous ABA and enhancement of abscission of flowers and reproductive organs. Spraying cowpea plants with all applied concentrations of IAA exerted marked changes in endogenous phytohormones during flowering as well as at the time of flower abscission. The treatments increased endogenous IAA; the increase was inversely propotional to the applied concentration. Decrease in flower abscission percentage accompanied by increase of endogenous IAA may be attributed to reduction of sensitivity to ethylene exerted by high level of endogenous IAA (Guo and Shang 1999). Endogenous cytokinins were found to be an effective factor in delaying senescence and decreasing flower abscission of soybean (Kitsaki *et al* 1999, El-Saeid *et al* 2010).

The balance between endogenous levels of promoters and inhibitors control abscission process. Sebanek and Klicova (1998) reported that auxins retard abscission of different organs. In chickpea the effects of ethrel unlike other PGRs viz., benzyladenine, triadimefon primarily was not related to increase in number of reproductive units and the difference in this behavior could be attributed to the concentration and stage dependent effect on flower and pod retention (Saxena *et al* 2000, 2003). An examination of the ethylene sensitivity and responsiveness of *Alstroemeria* flowers using a range of techniques has shown that this species senesces independently of endogenous ethylene production, but the completion of abscission requires a small burst of ethylene in the previous 24 h prior to cell separation (Wagstaff *et al* 2005). El-Saeid *et al* (2010) reported that increase of phytohormones (IAA, Gibberellins and cytokinins) concentration with decrease of ABA plays a role in decreasing percentage of flower abscission in cowpea.

Wanyama *et al* (2006) proposed that promotion of fruit setting on application of growth regulators was probably due to increased leaf expansion as a result of cell enlargement, which could have resulted in increased rate of photosynthesis; hence increased accumulation and partitioning of photosynthates thereby leading to increased fruit set. Reddy *et al* (2009) proposed that regular application of micronutrients along with major nutrients is essential for sustainable yield in pigeon pea, as rainfed soils are highly vulnerable to nutrient deficiencies. This study indicates that application of micronutrients like Mo, Fe, B along with N, P_2O_5, K_2O and S to soil before sowing enhances the productivity of pigeon pea in vertisols.

Abscisic acid (ABA) has been given wide attention for its role in controlling reproductive organ abscission. It was identified by Steveninck (1957, 1958) as the compound in lupin tissue which induced abscission in leaf explants. Abscisic acid has been linked with cotton (Davis and Addicot 1972) and peach fruit abscission (Martin and Nishijima 1972). However, the role of ABA in regulating abscission in bean and soybean is conflicting. Tamas *et al* (1979) found in two bean cultivars, Redkloud and Redkote, that removing older fruits growing at the base of a raceme reduced abortion of younger, distal pods and also lowered their ABA concentration in comparison to intact controls. The authors suggested that older fruit promote abscission of young 10 fruit at upper positions by increasing their level of ABA. In another study, Subhadrabandhu *et al* (1979) found contradictory results regarding an association between ABA and pod abscission in bean. In the bean cultivar Seafarer, ABA content of late and distally positioned pods was directly correlated with pod abscission, whereas in 'Black Turtle Soup' it was inversely related to abscission. However, removal of early flowers from both cultivars reduced ABA content and abscission of subsequently formed fruits. These authors concluded that ABA does not regulate fruit abscission in beans.

IAA promotes the prevention of pod abscission and cell elongation and suppression of pod abscission is the major determining factor of seed yield. On the other hand, auxin indirectly controls the ethylene activity, which accelerates the abscission. It also suppresses the cellulase activity, cell degrading enzyme which favours abscission process (Tekale *et al* 2009).

Spollen *et al* (1986) found removal of proximal soybean pods had no effect on the amount or concentration of ABA in seeds of distal pods but it did increase the ABA content in pod walls. Diethelm *et al* (1988) reported that the ABA content in abscising and non-abscising *Vicia faba* flowers was similar, and although removal of older pods resulted in less abscission among distal flowers, it did not affect their ABA level. Huff and Dybing (1980) also found that ABA did not mimic the effect of the unknown abscission-inducing substance extracted from proximal pods. Similarly, when ABA was injected into flower bearing nodes of lupin, no increase in the abscission rate was obtained at any position in the inflorescence (Porter 1977).

2.4 Dry matter

Total dry matter accumulation is one of the factors that determines economic yield in crop species where the seed is of economic importance. Leaf is the major organ where most of the photosynthates are produced. The number of leaves and their arrangement on main stem and side branches determine the structure of crop canopy which ultimately decides the dry matter production. The dry matter production at each growth stage and its partitioning to reproductive organs during pre-flowering to maturity period has immense importance in determining the final productivity.

Cycocel is an anti-gibberellin dwarfing agent, and foliar spray of this may induce deficiency of gibberellins in the plant and reduce the growth by blocking and conversion of geranyl pyrophosphate to coponyl pyrophosphate which is the first step of gibberellins synthesis. Significantly higher number of branches, dry weight and leaf area index was observed with bioregulators over the control (Moore 1980). Ravinchandran and Ramaswami (1991), also indicated that the application of mepiquat chloride, cycocel and TIBA significantly increase the amount of dry matter production in soybean. Growth regulators at higher concentrations showed decreased dry matter accumulation and yield, which could be attributed to the inhibition in metabolic pathways.

Mehetre and Jamadagni (1996) studied the biomass partitioning and plant architecture in 41 soybean cultivars and reported that the soybean plant has a tendency to deposit 19.77, 8.05, 29.42, 30.85 and 11.41 per cent assimilates into seed, pod wall, stems, leaves and roots, respectively. Progibb (4%), a gibberellin based vegetative growth promoter increased leaf length and dry weight of leaves in cilantro. Progibb (100 µl/l) was optimum for maximizing leaf production (Ramcharan 2000). Li and Solomon (2003) reported that biomass productivity per unit area increases in sugarcane following ethephon treatment. Resmi and Gopalkrishnan (2004) reported that foliar application of CCC (300, 400 or 500 ppm) to cowpea plants increased vegetative growth, fruit set and grain yield.

Kumar *et al* (2006) studied the influence of plant growth regulators on determinate (JS- 335) and semi- determinate (MACS-124) soybean genotypes and revealed that the growth retardants TIBA, mepiquat chloride and cycocel increased total dry matter production and biomass duration in both the soybean genotypes. They were more beneficial in terms of translocation of photo-assimilates towards developing reproductive parts as compared to growth promoter, kinetin and control. Emongor (2007) observed that GA_3 applied cowpea plants had significantly higher dry matter content in whole plant, shoot and root than control plants. The response of cowpea cv. Blacke to increasing GA_3 concentration was quadratic with respect to dry matter accumulation. Similarly, Ibrahim *et al* (2007) revealed that GA_3 (100 ppm) application led to increase in plant height, average number of leaves, leaf area per plant and dry weight of shoot in *Vicia faba*. Kalyankar *et al* (2008) showed that foliar spray of GA_3 (15 ppm) increased number of leaves and leaf area. NAA (100 ppm) was effective in increasing total dry weight.

2.5 Growth Analysis

The technique of growth analysis has been extensively used for better understanding of the physiological basis of yield variation in crop plants. Growth analysis is a physiological probe on the development of the crop in a chronological sequence to elucidate and account the causes for differences in yield through the events that have occurred at different stages of growth (Krishnamurthy *et al* 1973).

Extensive studies have been made on the physiological analysis of growth parameters in cereals, pulses and oilseeds emphasizing their importance in yield analysis (Chhonkar and Singh 1959). Mehra *et al* (1987) also observed that morphological architecture contributes to partition greater share of assimilates into vegetative production till maturity, consequently the plants fails to achieve potentials w.r.t increase in yield. The findings underscored that a desirable plant type should have the reproductive sink more competitive at the time of flower flushes concomitantly higher LAI is also desirable during this phase.

Variation in dry matter accumulation and pod production in different genotypes may be related to some factors such as leaf area (LA), crop growth rate (CGR), net assimilation rate (NAR) and relative growth rate (RGR). Pandey *et al* (1978) analyzed growth parameters in five varieties of blackgram in order to study the physiological causes of yield differences and observed the differences in CGR, NAR, RGR and LA among the varieties. Egli and Zhenwen (1991) suggested that seeds per unit area were related to canopy photosynthesis during flowering and pod set and canopy photosynthesis rate is determined through LAI and CGR. A plant with optimum LAI and NAR may have higher biological yield as well as seed yield (Mondal *et al* 2007). The dry matter accumulation may be the highest if LAI reaches its maximum value within the shortest possible time (Khan and Khalil 2010).

Tesfaye *et al* (2006) reported that attainment of high LAI that reduces soil water evaporation intercepts and converts radiation into dry matter efficiently and partitioning of the dry matter to the seed is the major requirement of a high seed yield in grain legumes in semiarid environments. Karim and Fattah (2007) reported that LAI, NAR and CGR were increased to pod filling period, LAD was decreased to first pod setting and biomass was increased all vegetation period in chickpea. Özalkan (2010) found that grain yield was significantly positive correlated with biomass, LAI and CGR at linear vegetative stage and with LAI, LAD at flowering stage. Also among the growth parameters there were found positive correlations.

Balkrishnan and Natarajarathan (1987) studied the aspects of critical leaf area index in pigeonpea. They found that LAR, NAR and CGR decreased progressively from the first to third cropping season. Average LAI increased rapidly between 50 DAS and first flowering, CGR was highest between 50 DAS and first flowering and reached a peak of 6.12 at 50 percent flowering, while NAR declined from 50 DAS until harvest. The critical LAI was considered to be 5.3, coinciding with maximum CGR and NAR.

Specific leaf weight (SLW) is a stable character and was initially low and improved subsequently and reached the maximum value at 42 days in greengram genotypes (Kalubarme and Pandey 1979). Crop productivity is mainly determined by crop growth rate (CGR) which depends on leaf area index (LAI) and the rate of photosynthesis. The CGR was found to be less during early vegetative stage but increased with the advancement of growth in greengram (Kalubarme and Pandey 1979). They further reported that NAR and RGR decreased with the advancement of crop growth. Nijhawan and Chandra (1980) observed wide variations in leaf area among different mung bean varieties and higher leaf area duration (LAD) during bud initiation stage was found to be obligatory for better yield. In another study, Misra and Dwivedi (1980) found that ragi seeds treated with potassium and distilled water produced significantly higher leaf area and dry weight of shoot compared to control. Henry and Gordon (1980) noticed that the treatment of triacontanol to pea increased the plant growth like root and stem biomass.

Thirumalaiswamy and Sakharam Rao (1977) reported that seed treatment with distilled water, CCC (5 ppm) and kinetin (5 ppm) and moisture levels slightly increased net assimilation rate (NAR), relative growth rate (RGR) and leaf area in pearlmillet and irrespective of moisture levels, the size of the leaf was greatly influenced by cycocel followed by kinetin.

Thirumurugan (2000) revealed that the growth parameters *viz.,* LAI and dry matter production in greengram were significantly higher with the foliar spray of KNO_3 (1%) or KCl (1%) and their combination. The foliar application of triacontanol (0.2 %) at vegetative, flowering and poding stages significantly increased the biomass production and better dry matter partitioning towards the sink in mungbean (Reddy *et al* 2002). Prakash *et al* (2003) reported that combined foliar application of planofix (30 ppm) at 30 and 45 DAS and chamatkar (120 ppm) at 60 DAS recorded higher LAI and SLW in blackgram. According to Sumeria (2003) the application of 60 kg P/ha and triacontanol granules at 30 kg/ha significantly increased growth parameters in mustard.

Patil and Dhomne (1998) reported that foliar application of growth retardants like CCC, TIBA, PCB each at two concentrates revealed an increase in growth parameters *viz.,* CGR, RGR, NAR and LAI. The growth parameters showed significant difference among the treatments and the rates fluctuated with growth and developmental stages of plant. Jadhav (2000) stated that the application of increasing concentrations of GA_3 and NAA increase the morphological and physiological parameters like CGR, RGR, NAR and LAR in soybean which inturn led to the increased yield and yield attributes. Similarly, Sarkar *et al* (2002) showed that double spraying of GA_3 and IAA (100 ppm) at 20 and 42 days after sowing increased LAI, CGR and NAR in soybean (cv.BS-3). The foliar spray of GA_3 (100 ppm) at 30

DAS had the most regulatory effect to enhance root, stem, leaf and total dry matter, LAI, CGR, RGR and NAR in soybean (cv. PB-1) (Rahman *et al* 2004).

2.6 Yield and its attributes

Grain yield is the ultimate economic product of the crop, which is determined mainly by number of flowers per plant, the percent podset, the number of seeds per pod and seed size in mungbean [*Vigna radiata* (L.) Wilczek] (Mondal *et al* 2011a). Most of the yield components show direct influence on grain yield. In legume crops, many flowers are produced, but only a few set pods. Degree of flower shedding varies between 60-92% in soybean (Nahar & Ikeda 2002; Saitoh *et al* 2004), 70-90% in mungbean (Mondal *et al* 2011a), 80-91% in *Vigna unguiculata* (Hossain *et al* 2006) and 80-95% in *Cajanus cajan* (Fakir *et al* 1998, Begum *et al* 2007).

Agricultural application of vegetal bio-regulators is becoming a useful practice that has improved the yield of beans, corn and soybean (Vieira and Castro 2004). Application of bio-regulators is reported to improve photosynthetic activity and its assimilation leading to modification of various metabolic and physiological processes in plants and improving source-sink relationship which ultimately improved flowering, fruit setting, grain filling and test weight in different crops (Tekale *et al* 2009).

Ethrel has been reported to improve productivity of pulse crops like cowpea, pigeonpea, mungbean and soybean by increasing the number of pods, seed weight and seed yield (Chandra 1985, Bora and Bahra 1989). In cucurbits ethrel application at 250 ppm promoted pistillate flower formation (Robinson *et al* 1970). Devi *et al* (2011) reported that application of Ethrel @ 200 ppm gave significantly higher yield (1.75 t/ha) over the other treatments when applied at both flower-initiation and pod-initiation stages in soybean. The increase in the yield could be a reflection of the effect of bioregulators on growth and development, it might be due to (a) marked increase in the number of branches per plant which gave a chance to the plant to carry more flowers, pods and hence more seeds (b) marked increase in the photosynthetic pigments content, which could lead to increase in photosynthesis, resulting in greater transfer of assimilates to the seeds and causing increase in their weight. Maity and Bera (2009) observed that BR increased plant height, number of nodes/ plant, number of branches/plant, number of leaves/plant but SA noticed poor influence over these growth parameters in comparison to BR. However, both these PGRs increased flower production, number of pods set/plant and yield of green gram.

Ethrel (0.02%) + 80 kg N ha^{-1} treatment enhanced leaf area index, net photosynthetic rate, nitrate reductase activity, plant biomass by 75.5, 56.4, 72.1 and 39.1%, respectively, while pod number plant^{-1}, 1000-seed weight and seed yield were enhanced by 27.2, 5.6 and 71.9%, respectively (Mir *et al* 2008). Birbal *et al* (2009) found that significantly maximum total tuber yield (37.0 tonnes/ha) with GA_3 @ 200 ppm treatment, followed by ethrel (35.0 tonnes/ha) in comparison to control (30.4 tonnes/ha).

Hilmy and Gad (2002) showed that cobalt at 25 mg/kg soil had a positive effect on parsley plant growth, yield as well as chlorophyll content. Tiwari *et al* (2003) revealed that spraying of bioregulators appeared to be more beneficial than root dipping. Spraying of ethrel (25 ppm) gave highest bulb yield and plant height in onion. Further, they observed that application of GA (50 ppm) produced maximum number of leaves per plant both as spray and root dip.

Sinha *et al* (2002) found that ascorbic acid treated moong and pea plants showed an increase in the nodule number and dry weight of the plants as compared to the control. Patil *et al* (2005) noticed that spraying of NAA (25 ppm) recorded significantly higher flower production and clusters per plant and total dry matter in mungbean. Similarly, Thakare *et al* (2006) found that foliar application of NAA (50 ppm) significantly increased the dry weight of pods per plant in soybean.

Associations between seed number and canopy photosynthesis, CGR during flowering and pod set suggest that photosynthesis and supply of assimilates mediates the relationship between environment and seed number (Vega *et al* 2001, Egli and Bruening 2002). Padi (2003) suggested that grain yield improvement in pigeonpea should be rapid if selection is made for a rapid crop growth rate, high harvest index and high no. of pods per plant. Each of these traits show a large positive direct effect as well as indirect effects through each other on grain yield.

Gupta (1984) suggested that the so called growth retardants suppress the vegetative growth if applied at flower initiation or ready to flower stage in legumes with indeterminate growth habit and thus, minimize the unproductive competition between vegetative and reproductive growth ultimately leading to economic yield improvement. Buttar and Aggarwal (2004) reported that various growth retardants such as pix (mepiquat), cycocel (chlormequat), alar (daminozide) and ethrel (ethephon) have found to increase oil and protein content in seed, 100-seed weight and seed cotton yield.

Seed number in soybean is affected by treatments that affect photosynthesis such as shade, increase in atmospheric CO_2 concentration, water stress and defoliation (Andrade and Ferrerio 1996, Board and Tan 1995). Sharma *et al* (2003) noticed that increase in seed yield in pigeon pea was due to significant reduction in plant height and increase in number of primary and secondary branches and pods per plant.

Raja *et al* (2009) inferred that though mepiquat chloride and ethrel sprays hastened flowering earlier than others, the important flowering characters, namely, number of inflorescences, number of female flowers, number of male flowers to one female and fertility coefficient were effectively enhanced by gibberellic acid and brassinolide treatments.

Saxena *et al* (2007) noticed that in chickpea the percentage of flowers and pods shed in treated plants in comparison to control was more when plants were sprayed with ethrel at post flowering stage than pre-flowering and mid-flowering stages. This happened because spray made at flowering stage is already past the stage showing any effect on plant. On the other hand, ethrel application at this stage might have hastened the senescence process. Hence, decrease in seed yield by ethrel application at post flowering stage is associated with high abscission of flowers and pods. The total number of pods formed at pre-flowering and mid flowering stages show substantial increase over control with low and medium concentration, while high concentration registered a decrease.

Thus, growth regulators particularly growth retardants used in appropriate concentrations at appropriate time increase the yield either by altering dry matter distribution in the plant or by regulating growth. Reddy *et al* (2009) proposed that in cowpea increase in yield due to growth retardants could be attributed to an increase in percent distribution of pod dry weight, increase in leaf thickness (SLW) and increase in pod length, 100 seed weight and total dry matter production.

Cobalt is required in low levels for maintaining high yields of tomato (Renner *et al* 2003) groundnut (Basu *et al* 2006) sweet potato (Gad and Kandil 2008) and potato (El-Bordiny and Gad 2008). Ngatia *et al* (2004) reviewed that in pigeonpea and broadbean high application of GA_3 led to increase in podset, no. of fruit bearing nodes, pod weight and seed yield.

Pujari *et al* (1998) studied the foliar application of urea and triacontanol and reported a significant increase in seed yield of pigeonpea. Similarly, Govindan and Thirumuragan (2000) observed that the foliar spray of KCl (1 %) + KNO_3 (1%) to increase the grain yield of greengram by 21.8 per cent over control. Reddy *et al* (2002) revealed that the foliar application of triacontanol (0.2%) at vegetative, flowering and poding stages significantly increased seed yield in mungbean grown under rained condition. In another study, Upadhyay (2002) noticed that foliar spray of NAA (20 ppm) resulted in significantly higher number of flowers per plant, pod length, grain number per pod, number of pods per plant, biological yield and grain yield. The highly significant positive correlation between yield and no. of pods/plant indicate that yield is largely dependent on no. of pods/plant in pigeonpea (Padi 2003).

Garai and Datta (2003) found that nodules per plant and yield increased due to foliar application of cycocel in greengram. Combined foliar application of planofix (30 ppm) at 30 and 45 DAS and chamatkar (120 ppm) at 60 DAS recorded increased number of pods per plant, number of seeds per plant, 100 seed weight and yield in blackgram (Prakash *et al*

2003). Similarly, Kalpana and Krishnarajan (2003) reported increased the number of pods per plant, number of filled seeds per pod, total number of seeds per pod, seed filling percentage, test weight and yield in soybean due to the foliar application of NAA (40 ppm).

Cho *et al* (2002) found that Low 2,4-DP (0.04 mM) and BAP (0.5 mM) in two soybean genotypes (Pungsan and Manlee) significantly reduced flower abortion and delayed abscission of pods , resulting in increased pod setting rates. Under field conditions using intermediate concentrations, 1 mM BAP significantly increased 100-seed weight to 22.3 g at R_1 in Manlee and 11.9 g at R_3 in Pungsan. BAP (1 mM) at R_3 in Pungsan significantly improved seed yield (40.1 g plant^{-1}). Maturity was not significantly affected by either application in Manlee, but was significantly affected by BAP in Pungsan.

Resmi and Gopalkrishnan (2004) reported that NAA (15, 30 or 45 ppm), 2, 4-D (2ppm) and CCC (300, 400 or 500 ppm) increased seed yield, pod length, pod weight, pod number per plant and pod number per unit area of cowpea plants. Tickoo *et al* (2006) showed that pre-sowing seed treatment with TIBA (10-30 ppm) and 2,4-D produced maximum seed yield in mungbean varieties *viz*., Pusa Vishal, Pusa 9072, Pusa 9531 and PS-16. This was due to more positive contributions by high HI, 100-seed weight, seeds per pod and pods per plant towards grain yield. Salunkhe *et al* (2008) studied the influence of plant growth regulators *viz*., TIBA (100 ppm), NAA (50 ppm), GA_3 (50 ppm), CCC (500 ppm), CCC (1000 ppm) on soybean cultivars (JS-335 and Phule Kalyani). Among the PGR treatments NAA (50 ppm) was found to be the best. The variety JS 335 recorded higher grain yield (q ha^{-1}) than Phule Kalyani and was found to be promising in major yield contributing characters and morphological traits.

Vasudevan *et al* (2008) revealed that interaction effect between apical bud pinching and GA_3 sprays showed a significant influence on growth, seed yield and yield attributes like number of pods per plant, number of seeds per pod *etc.* Kalyankar *et al* (2008) observed that CCC (500 ppm) was effective to increase number of grains/pod, 100-seed weight and grain yield in soybean variety MAUS 61-2.

2.7 Biochemical studies

The viable developmental changes are based upon the occurrence at biochemical level. During pod development there are shifts in the level of nitrogenous compounds, carbohydrates and enzyme activity. The partitioning of nitrogen in pigeonpea was dependent on growth habit, sensitiveness to nitrogen fixation and differences in leaf water potential of the genotypes which was significantly influenced by drought (Nandwal *et al* 1992).

Koti (1997) observed significant variations in chlorophyll content in determinate and indeterminate cultivars of soybean. While, Dhopte and Suradkar (1998) conducted a field experiment to study the effect of hormones (GA and NAA 20 ppm each) in soybean and found that the dry matter production, chlorophyll and root nodule numbers remained unaffected. Among hormones, NAA was more effective than GA. Tagade *et al* (1998) studied the influence of PGRs by soaking seeds of soybean in 25-150 ppm IAA and kinetin before sowing and noticed that leaf chlorophyll and nitrogen contents, seed yield and seed protein and oil contents increased with IAA concentration upto 100 ppm then decreased with increasing concentrations. While, Bora and Sarma (2004) showed that chlorophyll content decreased at higher GA_3 concentration while it was increased by CCC. Increase in chlorophyll content and nitrate reductase activity due to growth retardants might also have contributed for increase in yield and yield components (Reddy *et al* 2009).

Ibrahim *et al* (2007a) observed that the foliar application of growth retardant ancymidol on faba bean caused a significant increase in the content of photosynthetic pigments. The application of growth retardants and nipping at 35 DAS in cowpea increased the chlorophyll content. The maximum chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll contents were recorded in maleic hydrazide and lihocin at higher concentrations (Reddy *et al* 2009).

According to Taiz and Zeiger (2006), cytokines promote the development of chloroplasts and inhibit the chlorophyll degradation and, thus, prolong the plant photosynthetic period which will contribute to the production of soybean seeds. Gulluoglu *et al* (2006) proposed that the ethephon may have delayed the phenological cycle of the crops and the treatment with mepiquat chlorite + IBA + BAP also maintained the chlorophyll content high in the end of the crop cycle, which reinforces the fact that cytokines inhibit the degradation of chlorophyll on plants. The non-degradation of chlorophyll on the tissue maintain them photosynthetically active, thus, it provided larger quantity of organic matter to the grain filling, leading to the formation of heavier grains, and, consequently, increasing grain productivity (Campos *et al* 2010). Hajouj *et al* (2000) reported that cytokinin delay the initiation of leaf senescence and bring about the promotion of photosynthetic activity mainly by increasing the chlorophyll content.

Grewal *et al* (1993) reported that cycocel improves the translocation of photosynthates. More protein content stored in the seeds might be due to improvement of translocation of photosynthates to the seeds. Goyal and Ramawat (2008) found that ethrel treatment enhanced isoflavonoids accumulation in cell suspension cultures of *Pueraria tuberose*. The increase was dose dependent and can be explored to trigger high yield of isoflavonoids production.

Nitrate is the main source of nitrogen for crop plants. Its uptake is energy dependent and is mediated by a carrier on the plasma membrane of root cortical cells and the process is temperature sensitive. Crop species differ in the extent of nitrate assimilation in the roots versus the shoots. The enzyme nitrate reductase regulates the assimilation of nitrate. It is ubiquitous but the highest specific activity is usually found in the leaves. Though the enzyme activity is affected by the developmental changes, the overall pattern is determined by substrate level. Correlation between nitrate reductase activity, seed yield and seed protein content has been reported by Nair *et al* (1993).

Nitrate reductase (E.C. 1.6.6.1) mediates the reduction of nitrate to nitrite, which is regarded as a rate limiting step in plant growth and development (Solmonson and Barber 1990). Nitrate reductase activity (NRA) provides a good estimate of the nitrogen status of plants and is correlated with growth and plant yield (Srivastava 1980). Deckard *et al* (1973) found that NRA in the total leaf canopy of corn correlated positively with grain protein, grain yield and total reduced nitrogen (N) in the vegetative material at maturity. Eilrich and Hageman (1973) found a significant positive correlation between NRA and grain N and grain yield in wheat.

Degradation of leaf proteins during foliar senescence is required in order to remobilize leaf nitrogen and export it to developing grain and other sinks (Feller and Fischer 1994).When demand from sinks is not met by current assimilation, stored N is remobilized. Once low molecular weight reserves have been drained, polymers begin to be catabolized. Leaves, and particularly chloroplasts, are sites of protein accumulation. Most of the mobilizable protein in a leaf is soluble. Chlorophyll, on the other hand, is strictly confined to plastid membranes. The chlorophyll-protein relationship is much closer for membrane proteins than it is for soluble or total leaf proteins (Thomas *et al* 2002).

The enzyme nitrate reductase is cytoplasmic, containing molybdenum and flavin co-enzyme, FAD. Wasnik and Bagga (1992) showed that there was no effect of CCC (500 ppm) foliar spray on NRAse activity in greengram. Kumar *et al* (1999) reported that salicylic acid (SA *viz*; 0, 25, 50, 75, 100, 125 and 150 ppm) when sprayed on soybean at 12, 24 and 36 days after sowing accelerated the nitrate reductase activity and enhanced the content of total soluble proteins.

Antony (1995) reported that nitrate reductase activity was correlated with TDM at early stage but did not have positive correlation with any of the yield and yield components in groundnut. Kumaravelu *et al* (2000) indicated that foliar spray of 0.5 and 1.0 mg dm^{-3} triacontanol increased the nitrate reductase activity in greengram. Muthuchelian *et al* (1994) indicated that foliar spray of triacontanol significantly increased the nitrate reductase activity in *Erythrina variegata* seedlings. Wasnik and Bagga (1992) reported that the application of cycocel @ 500 ppm increased the nitrate reductase activity in greengram leaves. Sivakumar *et al* (2002) indicated that foliar application of triacontanol @ 10 ppm significantly increased the nitrate reductase activity at 60 DAS in pearl millet. Foliar application of cycocel with 3000 ppm to sunflower at 33 and 53 days after sowing decreased the nitrate reductase activity during growth stages, while 5000 ppm chlormequat decreased nitrate reductase activity throughout all growth stages (Pando *et al* 1988).

Khan (1996) reported impressive increase in the nitrate reductase activity (NRA) of mustard leaves in response to GA_3 application at optimal basal of nitrogen. At basal 80 kg N/ha, ethrel 200 µL/L significantly increased the nitrate reductase activity in leaves of *Brassica juncea* (Lone *et al* 2010).

Studies made by Singh and Misra (2001) on effect of GA_3 and ethrel at high concentration (1000 microgram/m) on *Mentha spicata* var. M35-5 observed enhancement in fresh weight biomass, leaf stem ratio, specific leaf weight and chlorophyll content. Nitrate reductase activity and peroxidase activity with low oil content. Comparison of peroxidase activity in pedicels and ovaries of abscising and non-abscising reproductive structures showed that activity was higher in samples from abscising structures (Ganpat 1989).

Researchers at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India have developed high protein lines (HPL) with up to 32.5% protein content and significantly higher sulphur-containing amino acid (cysteine and methionine) (Singh *et al* 1990, Saxena *et al* 2002). Pigeonpea is therefore a good source of amino acids (Elegbede 1998). A study on electrophoretic changes in seed proteins of *Vigna mungo* revealed rapid onset of storage protein accumulation from 43 days after sowing till maturity (Kaur 1999). Ghai *et al* (2008a) in their studies indicate that albumin synthesis increased rapidly till 24 days after anthesis (DAA) and thereafter though the increase was less steep after 24 DAA. In another study the application of homobrassinolide and humicil resulted in increase in level of sugars, amino acids and enhanced deposition of reserves in the form of proteins, which results in improved quality of mashbean seeds (Ghai *et al* 2008b).

Brassinosteroids might be working as 'metahormones' by triggering the changes in cellular sensitivity to plant growth regulators, thereby resulting in improved seed quality (Amzallag 2002). Higher protein content in green gram seed appears to be due to uptake and mobilization of minerals for enzyme activity involved in biosynthesis of major amino acids which have strong background for enhancing protein content of seeds (Poole *et al* 1983).

Invertases cleave sucrose to glucose and fructose. They are classified on the basis of stability, cellular location, and pH optimum. Based on pH optimum, acid invertase and neutral invertase were present. Invertase activities generally peak later than sucrose synthase activity during fruit growth, which is related to enlargement of sink cells, and usually have a sharp rise during the ripening process (Johnson *et al* 1988, Yelle *et al* 1988), which seems related more to ripening or after-ripening of tomato fruits. Johnson *et al* (1988) reported a constant stable acid invertase activity during most of fruit development, regardless of fruit growth rate, and then a six fold increase of activity at ripening whereas Yelle *et al* (1988) found a single peak curve with highest acid invertase activity at mid fruit development. It seems that the sharp rise in invertase activity at maturity may be related to the ripening process in tomato fruits. They noted low levels of invertase activity that decreased as fruit matured and no detectable sucrose synthase throughout fruit development.

Increasing activity of phenylalanine ammonia lyase in the pod wall of soybean increased pod shattering. Application of trans-cinnamic acid and $CoCl_2$ decreased the phenyl alanine ammonia lyase activity and reduced pod shattering (Gulluoglu *et al* 2006). Senthil *et al* (2005) conducted experiment to study the effect of growth regulators on IAA oxidase, peroxidase and NRase activities in groundnut under different salinity levels and indicated that seed treatment with GA_3 and IAA solutions reduced the activity of IAA oxidase and increased the activity of NRase enzyme. Similarly, Reddy *et al* (2009) showed that nitrate reductase activity increased upto 60 DAS and then decreased.

Soluble sugars such as fructose and glucose, made available directly by photosynthesis or indirectly via the hydrolysis of sucrose (transport form), are the main substrates for respiration (Saglio and Pradet 1980). According to Couée *et al* (2006) soluble sugars play a pivotal role in plant structure and metabolism at the cellular and whole plant levels. The authors further maintained that various metabolic reactions and regulatory compounds directly link soluble sugars with mitochondrial respiration or photosynthesis regulation and conversely with anti-oxidative processes such as the oxidative pentose phosphate pathway. It is very important to understand that a photosynthesizing plant regulates the amount of carbohydrate stored in the form of sucrose, starch or fructans and this is dependent on supply or demand. Measuring sugar levels under research conditions can therefore be a handy indication of the potential energy status of a crop as well as the potential substrate available for normal grain filling and an acceptable yield (Pretorius and Small 1992). Equally, the respiration rate of plant tissues is related to substrate supply and demand as well as respiratory energy demand (Dwivedi 2000). Kalappanavar and Hiremath (2000) reported that the amount of total sugars decreased significantly with the age of the plants. Sharma *et al* (1991) reported that the application of phenolic compounds increased total free sugar content in plants.

Bera *et al* (2008) reported that high amount of sugar and starch in mature seeds of green gram by foliar application of BR and SA might be due to enhanced CO_2 fixation, induced activity of carbohydrate synthesizing enzymes coupled with effective partitioning of dry matter into reproductive sink. Sivakumar *et al* (2002) also reported similar results in pearl millet by foliar application with plant growth regulators. Amanullah *et al* (2010) reported that optimum concentration of plant growth regulators might enhance the translocation of sugars and starch from pod wall to the developing seeds.

Ranasinghe and Waidyanatha (2003) proposed that application of ethrel consistently increased the yield per day, yield per spadix, yield per palm per annum and sugar content in the sap for a period of four years. The removal of carbon, nitrogen, phosphorus, potassium and magnesium with the sap was greater from ethrel treated than from untreated palms and this was attributed mainly to the enhanced sap yields and sugar concentrations.

Total soluble sugar (TSS) content is not only the main photosynthate in higher plants, but also the main form of carbohydrate metabolism and temporary storage. Wilcox (2001) indicated that the soluble sugar content plays a very important role in carbohydrate metabolism and has a close relationship with photosynthesis and production. The level of soluble sugar content was a sign of the supply ability of leaves and reflected transformation and ability of grains to use assimilates (Saratha *et al* 2001). Wang *et al* (2007) reported that the photosynthate exists as a form of water soluble carbohydrate (WSC) and its main components are soluble sugar, starch and cellulose. Differences in water-soluble carbohydrates among soybean cultivars and fertility levels were relatively small (Egli *et al* 1980). Percentages of WSC in leaves increased throughout the growing season, while WSC contents in stems, petioles, and pods decreased as the seeds developed (Ciha and Brun 1978). This decrease may indicate that soluble metabolically active carbohydrate is a factor involved in seed production during the seed-filling period. Li and Solomon (2003) reported that ethephon application promoted sugarcane quality and sugar accumulation, which lead to increase both cane yield and sucrose content.

Rai *et al* (2008) observed increase in reducing sugars and a decrease in sucrose contents of sugarcane on treatment with potassium, zinc, and Ethrel. Acid invertase, adenosine triphosphatase, indoleacetic acid oxidase, and nitrate reductase in vivo activities were also enhanced. Singh *et al* (2002) found that ethrel @ 300 ppm greatly increased the colour of fruit and total soluble sugars in apple.

Erulan *et al* (2009) reported that Seaweed liquid fertilizer (SLF) at low concentrations enhanced biochemical parameters like chlorophyll ‘a’ and ‘b’, protein, sugars, starch, ascorbic acid and *in vivo* nitrate reductase activity were also found higher at 1.5%. The higher concentrations like 2, 2.5 and 5% appeared to be the inhibitory levels for *Sorghum* reported by Vijayanand *et al* (2004).

Braun and Wild (1984) also reported an increase in the photosynthetic parameters in BR-treated wheat and mustard plants because of the enhancement in CO_2 fixation. The photosynthesizing tissues of C_3 and C_4 plants contain carbonic anhydrase that makes CO_2 available to Rubisco by catalyzing reversible hydration of CO_2. The distribution pattern of CA is, therefore, very much comparable with that of Rubisco.

PEPC [PEP (phosphoenolpyruvate) carboxylase] (EC 4.1.1.31) is an important enzyme situated at a crucial branch point in plant carbohydrate metabolism. It catalyses the irreversible β-carboxylation of PEP in the presence of HCO^{3-} to yield OAA (oxaloacetate) and P_i using Mg^{2+} as a cofactor. The enzyme is present in all plants, green algae and cyanobacteria, most archaea and non-photosynthetic bacteria, but is absent from animals and fungi (O'Leary *et al* 2011).

2.8 Anatomy of Inflorescence

Pre-mature abscission of flowers is one of the most serious problem in pigeonpea (Fakir 1998). About 70-96% floral abscission occurs in pigeonpea. Yield of pigeonpea remains low due to high level of floral abscission. The flowers and pods of the inflorescence may not receive enough assimilates from the leaf due to inadequate phloem or tissue development. The reduction in the phloem tissues on the apex of inflorescence probably allows translocation of insufficient photosynthates to support few or no flower to set pods i.e., insufficient photosynthates supply in the top of the inflorescence contributed shedding of flowers and buds (Bari and Prodhan 2001).

Begum *et al* (2007) proposed that removal of flowers and buds from the basal three nodes the pods sets in 4-6 nodes of the same rachis. For upper formed pods, it needs mechanical support which is related to the economic yield. Therefore, the mechanical tissue becomes highly developed in the deflowered rachis. Large vessels and prominent sieve tube elements are found to be developed in the deflowered rachis to conduct water and food materials for the growth and development of upper formed pods.

Abscission zones are usually located at the base of petioles and pedicels where the organ is attached to the axis, and are frequently discernable externally as a constricted area (Addicot 1982). Anatomically the zone usually develops at the same time as the organ and is characterized by small brick shaped cells which may be two to many layers thick. There are two steps to the process; firstly a separation layer develops within the AZ and two cell layers including the separation layer move apart, and secondly protection of exposed surfaces occurs either as a result of periderm formation or suberisation. AZ cells can be identified physiologically before abscission initiation by their response to ethylene (Osborne *et al* 1985). This and other features suggest that abscission zone cells are biochemically as well as morphologically distinct before the separation process (Roberts *et al* 2000).

Miller *et al* (2010) observed constriction at the base of the rachis, rachilla and pedicel in pigeonpea indicated the position at which separation occurred. A typical abscission zone which develops at the time of organ formation or as a result of secondary cell divisions just prior to separation does not occur in pigeonpea rachis, lateral rachillae and pedicel junctions.

Rančić *et al* (2010) examined tomato deficient mutant and wild type to elucidate role of ABA. Fruits of the ABA deficient mutant are smaller as consequence of smaller-sized cells in pericarp since functional xylem area in fruit pedicels and diameter of xylem elements in both genotypes are similar. Wild type has more nonfunctional xylem that could serve as a mechanical support for heavier fruits. Lower fruit dry weight and phloem efficiency in pedicels of ABA deficient mutant compared to the wild type suggests important role of ABA in the fruit sink activity.

Chapter-III

MATERIAL AND METHODS

An investigation entitled “Effect of plant growth regulators on manipulation of source-sink relationships in pigeonpea (*Cajanus cajan* L.)” was carried out in the field area of department of Plant Breeding and Genetics and laboratories of department of Botany, PAU, Ludhiana. The seeds of pigeonpea (*Cajanus cajan* L.) varieties were procured from the Department of Plant Breeding and Genetics, PAU, Ludhiana.

Location

Ludhiana represents the Indo-Gangetic plains and is situated at 36^0-54’N latitude, 25^0-48’E longitude and at a mean height of 247 meters above sea level.

Experiment I

Physiological evaluation of twenty five pigeonpea (*Cajanus cajan* L.) genotypes for flowering and pod set.

3.1 Methodology

Seeds of pigeonpea (*Cajanus cajan* L.) genotypes were obtained from the Department of Plant Breeding and Genetics, PAU, Ludhiana. Twenty five genotypes characterized as early maturing (AL 1756, AL 1757, AL 1758, AL 1760, AL 1811, AL 1816 and AL 1817), mid- late maturing (AL 1578, AL 1593, AL 1692, AL 1702, AL 1743, AL 1744, AL 1770, AL 1778, AL 1794, AL 1798 and AL 1809) , late maturing (AL 1721, AL 1779, AL 1790 and AL 1792) and as check (AL 201, PAU 881 and P 992) were grown under normal field conditions with recommended package of practices using following standard plot size:

Plot size	:	4 × 4 m x 0.5 m= $8m^2$
Number of rows	:	4
Row length	:	4m
Row to row spacing	:	50 cm
Replicates	:	3
Design	:	Randomized block design

3.2 Raising of crop

The crop was raised during *Kharif* season of 2010-11 and 2011-12 in first week of june following the practices described in the package of practices (Anonymous 2009).

3.3 Observations

In this experiment, various observations with regard to the growth (vegetative, flowering and post flowering) and yield were recorded. Three plants were randomly selected and tagged in each plot. The details of observations recorded are as under:

3.3.1 Morpho-physiological parameters

3.3.1.1 Plant height

The length of the main stem was measured in cm from the ground level to the tip of the plant at vegetative, flowering and post flowering stages.

3.3.1.2 Number of branches plant^{-1}

The total number of branches were counted and recorded as number of branches per plant.

3.3.1.3 Growth efficiency

i) Relative growth rate (RGR)

RGR is defined as increase in plant dry matter per unit plant material per unit time. It is expressed as gram per gram dry weight per day.

$$RGR = \frac{1}{W} \times \frac{W_2 - W_1}{(t_2 - t_1)}$$

ii) Crop growth rate (CGR)

The dry matter accumulation rate per unit area is referred as crop growth rate (CGR), normally expressed as grams per square meter land area per day (g/ land area/ day). It can be calculated with the help of following formula:

$$CGR = \frac{W_2 - W_1}{[SA] \times (t_2 - t_1)}$$

Where,

W_1 and W_2 = crop dry weight at beginning and end of the interval

t_1 and t_2 = corresponding days

SA= soil area occupied by the plant in each sampling

3.3.1.4 Leaf characteristics

The following leaf characteristics were recorded at different stages:

i) Leaf area plant^{-1} (LA)

LA plant^{-1} was recorded by graph paper method and was expressed as cm^2 plant^{-1}.

ii) Leaf area index (LAI)

Direct values for leaf area index were recorded with the instrument "Sun –Scan Canopy Analyzer".

iii) Specific leaf weight (SLW)

After taking the leaf area these leaves were subsequently dried at 60°C and used for specific leaf weight (SLW) determinations. SLW calculation has been done as per following formula. Results were expressed as mg cm^{-2} plant^{-1}. Formula is as follows:

$$\text{Specific Leaf Weight} = \frac{\text{Leaf weight (mg)}}{\text{Leaf area (cm}^2\text{)}}$$

iv) Specific leaf area (SLA)

After taking the leaf area these leaves were subsequently dried at 60°C and used for specific leaf area (SLA) determinations. SLA calculation has been done as per following formula. Results were expressed as $cm^2\ mg^{-1}\ plant^{-1}$. Formula is as follows:

$$\text{Specific Leaf Area (SLA)} = \frac{\text{Leaf area (cm}^2\text{)}}{\text{Leaf weight (mg)}}$$

3.3.2 Phenological traits

i) Days to 50% flowering

The days to 50% flowering was recorded from the date of sowing to the date when 50% of plants bear flowers in a plot.

ii) Number of flowers produced

Three random plants were tagged in each plot from each replication for recording the flowering behaviour. Thus, there were total nine plants of a genotype.

iii) Days to maturity

Number of days taken to maturity was recorded from the date of sowing to the date when 80% plants showed maturity.

3.3.3 Partitioning

Three plants were collected from each plot at vegetative, flowering, post-flowering and maturity. The leaves, stem, flowers and pods were separated according to the stage these samples were dried at 70±2°C for a period of 48 h , till a constant weight was obtained and dry weights were recorded. From this data, dry matter partitioning towards different tissues was calculated according to the following formulae:

$$\text{Leaf partitioning coefficient} = \frac{\text{Leaf DM}}{\text{Total DM}} \times 100$$

$$\text{Shoot partitioning coefficient} = \frac{\text{Shoot DM}}{\text{Total DM}} \times 100$$

$$\text{Flower partitioning coefficient} = \frac{\text{Flower DM}}{\text{Total DM}} \times 100$$

$$\text{Pod partitioning coefficient} = \frac{\text{Pod DM}}{\text{Total DM}} \times 100$$

Field view of pigeon pea

3.3.4 Yield parameters

Five plants were tagged in each plot to record the data pertaining to the yield attributes at maturity

i) Pod weight per plant

Pods of each plant were weighed and their weights were recorded in grams.

ii) Pod setting percentage

Pod setting was measured according to the following formula:

$$\frac{\text{Number of pods}}{\text{Total number of flowers}} \times 100$$

iii) Seed weight per plant

Seeds of each plant were weighed and their weights were recorded in grams.

iv) 100- seed weight

100-seeds from each treatment were counted and their weights were recorded in grams.

v) Seed yield per plot

The weight of seeds harvested from the respective plots was recorded and then converted in q/ha.

vi) Harvest index (HI)

$$\text{HI} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

EXPERIMENT II

Manipulation of reproductive efficiency of pigeonpea (*Cajanus cajan* L.) genotypes by the use of growth regulators.

Methodology

Four mid-duration pigeonpea genotypes from experiment I were selected on the basis of their genetic diversity with respect to flowering and pod set were grown in complete randomized block design as per Package of Practices for *Kharif*, 2009-10.

Foliar spray: The crop was foliarly sprayed twice with ethrel (100 and 200 $\mu g\ ml^{-1}$) and $CoCl_2$ (10 and 15 $\mu g\ ml^{-1}$) at vegetative and flowering stages and all spray treatments were repeated after two days. The foliar treatments as well as notations are summarized as follows:

- E_1V- Ethrel (100 $\mu g\ ml^{-1}$) at pre-flowering stage
- E_2V- Ethrel (200 $\mu g\ ml^{-1}$) at pre-flowering stage
- E_1F- Ethrel (100 $\mu g\ ml^{-1}$) at flowering stage
- E_2F- Ethrel (200 $\mu g\ ml^{-1}$) at flowering stage
- C_1V- $CoCl_2$ (10 $\mu g\ ml^{-1}$) at pre-flowering stage

- C_2V- $CoCl_2$ (15 μg ml^{-1}) at pre-flowering stage
- C_1F- $CoCl_2$ (10 μg ml^{-1}) at flowering stage
- C_2F- $CoCl_2$ (15 μg ml^{-1}) at flowering stage

No. of treatments = 4, control= 4, No. of genotypes =4, Stages = 2, replications= 3

Total number of plots = 120, No. of plants tagged in each plot= 3.

3.4 OBSERVATIONS

In this experiment, various observations with regard to the growth (vegetative, flowering and post flowering) and yield were recorded. Three plants were randomly selected and tagged in each plot. The details of observations recorded are as under:

3.4.1 Morpho-physiological parameters

(Morpho-physiological parameters as in experiment 1 were recorded).

3.4.2 Photosynthesis and related parameters

Net photosynthesis rate (Pn), stomatal conductance and internal CO_2 concentration was measured with a portable infrared gas analyzer (LI-6400XT, LICOR), following the method of Coombs *et al* (1985).

3.4.2.1 Total Chlorophyll Content (Hiscox and Israelstam, 1979)

Total chlorophyll content was estimated from 4th leaf at vegetative, flowering and post-flowering stages by Dimethyl sulphoxide (DMSO) method.

Reagent

i) Dimethyl sulphoxide (DMSO)

Procedure

The fresh leaves of 100 mg was cut into small pieces and put in the test tubes containing 5 ml of Dimethyl sulphoxide (DMSO). The test tubes were then placed in an oven at 60^0 C for about 2 hours or more to facilitate the extraction of pigments. After attaining required room temperature absorbance was read at 645 nm and 663 nm on a spectrophotometer and the chlorophyll content was determined using following equation:

$$\text{Total Chlorophyll} = 20.2\,(A_{645}) + 8.02\,(A_{665}) \times \frac{V}{1000 \times W}$$

Where,

A_{663} = Absorbance at 663 nm

A_{645} = Absorbance at 645 nm

V = Total Volume of the extract (ml)

W = Weight of the sample (g)

The chlorophyll content was expressed in terms of mg chlorophyll g^{-1} fresh weight.

3.4.3 Phenological traits

(as in experiment 1 were recorded).

3.4.4 Partitioning

(Partitioning parameters 1-4 as in experiment 1 were recorded)

3.4.5 Yield parameters

(Yield parameters 1-6 as in experiment 1 were recorded)

3.4.6 Biochemical parameters

3.4.6.1 Total soluble sugars (Dubois *et al* 1956)

Total soluble sugars were estimated from developing leaves at vegetative, flowering and post- flowering stages and from developing seeds from 10 DAF till maturity.

Sugars react with concentrated sulphuric acid to form a dehydration product i.e. furfural or 5-hydroxymethyl furfural. This dehydration product then reacts with phenol which acts as a chromphore and gives orange yellow colour.

Reagents:

i) 80% ethanol

ii) 5% phenol (redistilled)

iii) Concentrated H_2SO_4

Extraction: Samples of known weights were collected and homgenized in 80% ethanol and centrifuged. The residue was re-extracted to ensure complete extraction; supernatants were pooled and used for estimation of total soluble sugars. Residue left after ethanol extraction was kept for analyzing starch content.

Estimation: To 0.1 ml of sugar extract 1 ml of 5% phenol was added and kept for 10 minutes followed by addition of 5 ml of concentrated H_2SO_4. The sulphuric acid was poured directly in the middle of the test tube to ensure proper mixing of the solutions. After 10 minutes the tubes were cooled to room temperature under running water. After another 20 minutes the absorbance was measured at 490 nm against reagent blank. The concentration of total sugars was calculated from the glucose standards (10-60 µg) run simultaneously.

3.4.6.2 Starch (Dubois *et al* 1956)

Total soluble starch was estimated from developing leaves at vegetative, flowering and post-flowering stages and from developing seeds from 10 DAF till maturity.

Starch is hydrolyzed with the help of HCl and free sugars are released which then form dehydration product with concentrated sulphuric acid. This dehydration product then reacts with phenol which acts as a chromphore and gives orange yellow colour.

Extraction: The pellet left after extraction of sugars was retained and washed with 80% ethanol to remove all traces of total soluble sugars and dried at 60^{o}C. The pellet was then extracted for starch by boiling with 5 ml of 5N HCl. The extraction was repeated twice and supernatants were pooled to be used for starch estimation.

Estimation: To 0.1 ml of starch extract 1 ml of 5% phenol was added and kept for 10 minutes followed by addition of 5 ml of concentrated H_2SO_4. The sulphuric acid was poured directly in the middle of the test tube to ensure proper mixing of the solutions. After 10 minutes the tubes were cooled to room temperature under running water. After another 20 minutes the absorbance was measured at 490 nm against reagent blank. The concentration of total starch was calculated from the starch standards (10-60 μg) run simultaneously.

3.4.6.3 Total soluble proteins (Lowry *et al* 1951)

Protein in the sample reacts with copper tartarate complex in alkaline solution. The protein-copper complex then reduces phosphomolybdate of folin phenol reagent to form a blue-colour complex having maximum absorption at 620 nm.

Reagents:

A: 2% sodium carbonate in 0.1 N NaOH

B: 0.5% copper sulphate in 1% sodium potassium tartarate

C: 50 ml of reagent A mixed with 1 ml of reagent B (just before use)

D: Folin and Ciocalteau's reagent (2 N) diluted with water in 1:1 ratio

Extraction : The requisite quantity of the sample was ground in 5 ml of 0.2M phosphate buffer (pH 7.5), followed by centrifugation at 5000 g for 10 min. The extraction procedure was repeated twice and total volume was made to 5 ml. To 1 ml of the aliquot of protein extract, 1 ml of 20% trichloroacetic acid (TCA) was added and kept at 4°C for 24 hours. This extract was later centrifuged for 20 min at 5000 g and precipitates so obtained were dissolved in 0.1 N NaOH. Store the supernatant for estimating amino acids.

Estimation: To 0.1 ml of protein extract, 5 ml of reagent C was added and the mixture was constantly shaken and kept at room temperature for 10 min. To this 0.5 ml of reagent D was added and kept at 37°C for 30 min. The blue colour thus developed was measured at 570 nm against a blank. Protein was quantitized from the standard curve prepared by using bovine serum albumin (BSA). The protein content was expressed as mg protein g^{-1} dry weight.

3.4.6.4 Estimation of free amino acids (Lee and Takahashi 1966)

Reagents:

Ninhydrin reagent: Reagent was prepared by mixing the following constituents (A, B and C) in the ratio of 5:12:2

A = 1% ninhydrin in 0.5M citrate buffer (pH 5.5)

B= Pure glycerol

C= 0.5M citrate buffer (pH 5.5)

Extraction: 100 mg of dry material was taken and crushed with 3 ml of 80% ethanol followed by centrifugation. The extraction was repeated with 2 ml of 80% ethanol and total volume of pooled supernatant was adjusted to 10 ml.

Estimation: 5 ml of ninhydrin reagent was added to 0.1ml of ethanol extract. The contents were shaken vigoursly and the reaction mixture was boiled for 12 minutes in boiling water bath and then cooled at room temperature. Absorbance was recorded at 570nm with spectrophotometer against a blank of 80% ethanol replacing the extract. The standard curve was prepared using glycine (10-100 μg) as a standard amino acid.

3.4.6.5 Enzyme extraction and assays

All the enzymes were extracted with relevant extraction buffers at 4^0C to minimize denaturation and assayed at 25°C.

Frozen samples were homogenized in relevent buffer containing following reagents:

Reagents

A: Ethylendiamine tetraacetic acid (EDTA)

B: 2-Mercaptoethanol

C: 1% polyvinyl pyrrolidone (PVP)

The homogenate solution was centrifuged at 14000g for 15 min. and the clear supernatant was used directly for the assay of enzyme activity. Activity of enzyme was determined at 25°C with a spectrophotometer.

i) Nitrate reductase in leaves (E.C. 1.6.6.1)

Its activity was estimated by Jaworski (1971)

Reagents:

1. **0.1M Phosphate buffer (pH 7.5):** 72 ml of sodium hydrogen phosphate (0.312 g in 100 ml distilled water) was added to 28 ml of disodium hydrogen phosphate (0.355 g in 100 ml distilled water). The pH of the solution was adjusted to 7.5.
2. **0.5M KNO_3:** Dissolved 5 g of KNO_3 in 100 ml phosphate buffer.
3. **1% (w/v) sulphanilamide in 1.5 N HCl:** Prepared 1% sulphanilamide in 100 ml in 1.5 N HCl.
4. **0.2% N-(1-naphthyl) ethylene diamine dihydrochloride:** Dissolve 0.2 g solid reagent in 100 ml distilled water.
5. **Standard sodium nitrite solution:** 60 mg of $NaNO_2$ was dissolved in 100 ml distilled water to be used as stock solution.

Extraction: 200 mg of fresh plant material was cut to slices. Suspended the chopped plant tissues in a 5 ml reaction mixture which comprised of 1% propanol and 0.5 M KNO_3 in 0.1 M phosphate buffer (pH 7.5). Capped the test-tubes and incubated in dark at 30°C for 24 hours.

Estimation: After incubation 0.2ml aliquot of reaction mixture was removed and mixed with 0.1 ml of 0.2% N-naphthyl ethylene diamine hydrochloride (NEDH). After 20 minutes added 4 ml of distilled water to each tube. The absorbance was read at 570nm. The enzyme activity was expressed in μ moles NO^-_3 red h^{-1} g^{-1} FW.

ii) Phosphoenol Pyruvate (PEP) carboxylase (EC 4.1.1.32) Activity in leaves (Downton and Slatyer 1971)

Principle: Photosynthetic carbon-dioxide is fixed by PEP carboxylase enzyme and activity of this enzyme can be measured by using a coupled enzyme assay with malate dehydrogenase. Malate dehydrogenase enzyme reduces oxaloacetate to malate using NADH as the reductant which consequently is converted to its oxidized form in this reaction. The rate of formation of oxaloacetate and hence, the activity of PEP carboxylase can be monitored by recording the change in absorbance at 340 nm. The reactions involved in this coupled enzyme system are as follows:

$$\mathrm{PEP} + \mathrm{CO_2} \xrightarrow{\text{PEP carboxylase}} \text{Oxaloacetate} + \mathrm{P_i}$$

$$\text{Oxaloacetate} + \mathrm{NADH} \xrightarrow{\text{malate dehydrogenase}} \text{Malate} + \mathrm{NAD^+}$$

Reagents

A. Assay mixture contained 100 µ mole Tris HCl buffer (pH 8.0), 30 µ mole $MgCl_2$, 50 µ mole $NaHCO_3$ and 4.2 units of malate dehydrogenase (one unit is equal to one µ mole of NADH oxidized per minute).

B. NADH solution: 0.5 µ mole NADH solution was made in Tris-HCl buffer (pH 8.0).

C. PEP solution: 5 µ mole PEP solution was made in 100 µ mole Tris HCl buffer (pH 8.0).

Extraction: Leaf sample (100 mg) was hand homogenized at 0-4°C in a pre-chilled pestle and mortar using the extraction buffer containing 100 µM Tris-HCl (pH 8.0), 1% PVP and 1 mM EDTA. The resulting homogenate was centrifuged at 10,000 g for 20 minutes at 0-4°C. The supernatant so obtained was referred as enzyme extract.

Estimation: For the estimation of PEP carboxylase activity, 1.5 ml of assay mixture and 0.2 ml of suitable aliquot of the enzyme extract were taken and warmed at 37°C for about 2-3 minutes. Then 0.1 ml of NADH was added and decrease in absorbance at intervals of 15 seconds up to 3 minutes was recorded. This gave the rate of endogenous NADH oxidation and served as the blank. The reaction was initiated with the addition of 0.1 ml of PEP and the decrease in absorbance at 15 second intervals up to 3 minutes was recorded at 340 nm at UV spectrophotometer. The standard curve of NADH over a range of 0 - 0.3 µ moles prepared simultaneously. The enzyme activity was expressed as µ mol mg^{-1} protein min^{-1}.

iii) Invertase activity in developing seeds (EC 3.2.1.26)

The activity was measured by the method of Sumner (1935). Invertase catalyzes the breakdown of sucrose into glucose and fructose. The formation of the product is measured by heating with DNSA reagent.

Reagent

1. **0.1M acetate buffer (pH 5.0):** 14.8 ml of acetic acid (0.577 g in 100 ml distilled water) was added to 35.2 ml of sodium acetate (0.820 g in 100 ml distilled water). The pH of the

solution was adjusted to 5.0.

2. **0.2M acetate buffer (pH 4.8):** 20 ml of acetic acid (1.153 g in 100 ml distilled water) was added to 30 ml of sodium acetate (1.64 g in 100 ml distilled water) then diluted to a volume of 100 ml. The pH of the solution was adjusted to 4.8.
3. **DNSA reagent:** Dissolve 2.5 g of di nitro salicyclic acid (DNSA) in 50ml distilled water. Then add 4 g NaOH and stir well. Then dissolve 72 g potassium phosphate tartrate and make up 1 litre volume.
4. **Standard glucose solution:** 10 mg of glucose was dissolved in 100 ml distilled water to be used as stock solution.

Extraction

Invertase was extracted from tissue in 5 ml of 0.1M acetate buffer (pH 5.0). The tissue was crushed in pre chilled pestle and mortar and kept on ice. The extract was centrifuged at 10,000 rpm in a refrigerated centrifuge at 0^0 C for 15 min and final volume of the supernatant was made to 5 ml.

Estimation

The reaction mixture consisted of 0.4 ml acetate buffer (0.2M, pH 4.8), 0.2 ml sucrose (0.4M) and enzyme extract was added to give a total volume of 1 ml. In controls, sucrose solution was added after killing the enzyme by boiling for about 5 min in a water bath. Then 1 ml DNSA reagent was added to these tubes after incubating at 30^0C for 30 min and boil for 10 min. Allow it to cool and added 5 ml distilled water. Optical density was recorded at 560 nm. The enzyme activity was expressed in terms of reducing sugars formed from the reference curve prepared by heating varying amounts of anhydrous glucose (10-100 µg) with nmol DNSA reagent g^{-1} fresh weight min^{-1}.

3.4.7 Protein profiling in mature seeds (SDS-PAGE) (Walker 1996)

Extraction of proteins: 2.5 g of fresh tissue were taken in 2 ml of tris-HCl buffer (pH 6.8), the tissue were ground in pestle and mortar. The homogenized tissue was centrifuged in a cooling centrifuge at 4^0C for 20 min at 10,000rpm. Supernatant was collected as crude protein sample and stored at 4^0C.

A) Preparation of stock reagents:

1) Acrylamide-bisacrylamide solution: Dissolved acrylamide and N,N,N', N'-methylene bisacrylamide in the ratio of 30:0.8 in distilled water to prepare 100 ml solution. The mixture was filtered and stored at 4^0C in dark brown bottle.
2) 1.5M Tris-HCl buffer (pH 8.8): Dissolved 9.085 g of Tris-base in 25 ml distilled water. The pH was adjusted to 8.8 with 3N HCl and the final volume made to 50 ml with distilled water. The solution was stored at 4^0C.

3) Stacking gel buffer-0.5M Tris-HCl buffer (pH 6.8): Dissolved 3.963 g of Tris-HCl in 25 ml distilled water. The pH was adjusted to 6.8 with 1N NaOH and volume was made to 50ml with distilled water and solution was stored at 4 ^{0}C.
4) 10% (w/v) ammonium persulphate (APS): 10 mg of APS was dissolved in 100µl distilled water. A fresh solution was prepared every time before use.
5) 10% SDS: 1 g of SDS was dissolved in distilled water with gentle stirring and volume was made to 10ml.
6) TEMED
7) Sample buffer: mixed the following

0.5M Tris-HCl (pH 6.8)	:	2.5 ml
glycerol	:	2.5 ml
SDS	:	0.25g
2-mercaptoethanol	:	0.125ml
Bromophenol blue (0.1%)	:	2.5ml

Made volume up to 25ml with distilled water.

8) Reservoir buffer (pH 8.3): 3.0g Tris-base, 14.4g glycine and 0.5g SDS were mixed and total volume made to 500 ml with distilled water. This solution was stored at 4^{0}C.
9) Staining solution: Dissolved 0.1g Coomassie brilliant blue R-250 in 50ml methanol, 10ml glacial acetic acid and 40ml of distilled water.
10) Destaining solution: prepared by mixing methanol, glacial acetic acid and distilled water in the ratio of 100:70:830.

B) Preparation of resolving gel and stacking gel:

Reagents	Stacking gel (5%)	Resolving gel (12.5%)
Acrylamide: bisacrylamide	0.5ml	4.0ml
Tris-HCl	0.6ml	1.6ml
Distilled water	2.0ml	3.2ml
SDS	75 µl	0.1ml
APS	30 µl	60 µl
TEMED	8 µl	15 µl

C) Sample preparation and running of electrophoresis:

Protein samples were mixed with sample buffer in ratio of 1:1 and heated for 2 min to ensure complete dissociation and optimum SDS-binding. After loading the samples (20 µl in each), the electrophoresis was carried out at costant current of 1.5mA per cm, until tracking dye reached near the bottom of the slab gel.

D) Staining and destaining of gel:

The gel was stained by keeping in stain solution [0.1% Coomassie brilliant blue R-250 in methanol: acetic acid: water (50:10:40)] and then destained with destaining solution to visualize the protein bands. Stained gels were preserved for presence or absence of bands for comparison.

3.4.8 Anatomical studies

To study the structure of phloem of the control and treated plants in the pedicels of flowers (0 DAA), these were stored in FAA (formalin-acetic acid -alcohol) solution which was prepared according to Sass (1958).

Ethyl alcohol (95%)	50ml
Glacial acetic acid	5ml
Formaldehyde (90%)	10ml
Water	35ml

Dehydration

The fixed material was thoroughly washed in distilled water and then dehydrated using ethyl alcohol series which consisted of 10, 30, 50, 70, 90, 95% alcohol and two changes in absolute alcohol . In each change of ethyl alcohol, material was left for a period of 2h . This was then subjected to ethyl alcohol and tertiary butyl alcohol (TBA) series (3:1 ,1:1 and 1:3). Finally the material was brought to pure TBA and two changes of 6 hours each of pure TBA were given.

Infiltration

Infiltration of plant material was undertaken using paraffin-tertiary butyl alcohol (Para-TBA) mixture which was prepared by mixing equal volumes of molten paraffin and TBA. The flakes of para-TBA, which is solid at room temperature, were added to pass through tubes containing plant material at 58-62°C in the oven. More flakes of para-TBA were added at an interval of 4 hour for four days .Then flakes of pure paraffin were added for two days. After that the corks were opened to allow evaporation of TBA. Two changes of molten paraffin were given. The embedding of this material was undertaken in pure paraffin wax in the lid of coupling jar. Small rectangular blocks of the embedded material were mounted on wooden block for microtomy.

Microtomy

Serial sections of the plant material were cut on a rotary microtome at 10 μm thickness. Ribbons containing serials sections were mounted on glass slides using Haupt's adhesive which was prepared by dissolving 1g gelatin in 100ml of water at 90°C. This mixture was cooled to 30°C and then 15 ml of glycerine was added. 2 g of phenol was added as preservative (Jensen 1962) .To mount sections, slides were flooded with 2 percent formalin solution and ribbons were floated and spread properly. Excess of formalin solution from the slides was drained off on blotting paper. Slides with section in paraffin ribbons were kept at 40°C in the oven for drying.

Dewaxing

Before following staining producer, the slides were dewaxed using xylene. Two changes of xylene were undertaken at 2 hour each. The sections were hydrated using downward series of xylene: alcohol (3:1, 1:1, 1:3) and alcohol (absolute, 95, 70, 50, 30 and 10%) series. Then slides were brought to water and stained with erythrosine and crystal violet (Jensen 1962).

3.4.9 Statistical Analysis

i) Analysis of variance (ANOVA)

Analysis of variance for different characters was calculated using the ANOVA table for RBD

ANOVA table for RBD

Source of variation	d.f.	Sum of squares	Mean sum of squares	F- ratio
Blocks or replications	r – 1	$BSS = \sum_{i=1}^{r} \frac{y.j^2}{v} - \frac{y..^2}{rv}$	$MSB = \frac{BSS}{r-1}$	$F_1 = \frac{MSB}{MSE}$
Treatments	v – 1	$TSS = \sum_{i=1}^{v} \frac{yi.^2}{v} - \frac{y..^2}{rv}$	$MST = \frac{TSS}{v-1}$	$F_1 = \frac{MST}{MSE}$
Errors	(r – 1) (v – 1)	SEE (by subtraction)	$MSE = \frac{SSS}{(r-1)(v-1)}$	
Total	rv – 1	$SST = \sum\sum yij^2 - \frac{y..^2}{rv}$		

Where

r = no. of replications

v = no. of treatments

$yi.$ = i^{th} treatment total

$y.j$ = j^{th} block total

BSS = Block sum of squares

$y..$ = Total of all observations

TSS = Treatment sum of squares

SSE = Error sum of squares

SST = Total sum of squares

MSB = Block mean sum of squares

MST = Treatment mean sum of squares

MSE = Error mean sum of squares

ii) Correlation

The correlation of different characters was calculated taking two characters a time using the following expression.

$$r\,(X,Y) = \frac{\sum XY - N\overline{XY}}{\sqrt{\sum X^2 - N\overline{X^2} \quad \sum Y^2 - N\overline{Y^2}}}$$

Where

r = Correlation coefficient

X = one character

Y = second character

N = number of observations

$\bar{X}$ = sum of observations of one character/number of observations

$\bar{Y}$ = sum of observations of second character/number of observations

iii) Path Coefficient Analysis

Path coefficient analysis is simply a standardized partial regression coefficient. Path coefficient analysis permits partitioning of coefficient of correlation into direct and indirect effects. Path coefficient analysis was done following Deway and Lu (1959). The path coefficients were calculated by abbreviated ‘Do Little’ techniques.

$$Py_1 + Py_2.r_{12} + Py_3.r_{13} + \ldots\ldots + Pyn.r_1n = ry_1$$

$$Py_1.r_{12} + Py_2 + Py_3.r_{23} + \ldots\ldots + Pyn.r_2n = ry_2$$

$$Py_1.r_{13} + Py_2.r_{23} + Py_3 + \ldots\ldots + Pyn.r_3n = ry_3$$

$$Py_1.rn_{12} + Py_2 + Py_3.rn_3 + \ldots\ldots + Pyn = ry_n$$

Where, Py_1, Py_2, Py_3…. Py_n are the direct path effects of 1, 2, 3… n variables on the dependent variable ‘y’. r_{12}, r_{13} …. r_1n----r(n-1)n are the possible coefficients of correlation between various independent variables, and ry_1, ry_2, …. ry_n are the coefficients of correlation of independent variables with dependent variable ‘y’.

The variation in the dependent variable which remained undetermined by including the given variables was assumed to be due to variable not included in the present investigation. The degree of determination of such variables was calculated as follows:

$$\text{Residual Effect} = \sqrt{1\text{-}R^2}$$

Where,

$R^2 = Py_1.ry_1 + Py_2.ry_2 + \ldots + Pyn.ryn.$

R^2 is the squared multiple correlation coefficient and is the amount of variation in yield that can be accounted for by the yield component traits included in the study.

CHAPTER IV

RESULTS AND DISCUSSION

In view of the ever increasing national population and depleting natural resources (reduced cropping area and water supply in coming decades), a quantum jump in food production is the most desired goal. Our visionaries have enabled us to achieve the marvels of Green-Revolution by best organic practices, intensive land use and high yielding crop varieties. Any further demand of additional food production must be met by better and integrated management practices. This is particularly true in food sector, wherein increasing resistance to GMOs (Genetically Modified Organisms) and GM Foods are felt. Hence, there is an immediate need to enhance the yield potential of crops by managerial amendments. Although high yielding hybrid-crop varieties do extremely well under normal management practices, very seldom their full genetic potential is realised. Faced with such constraints, application of plant growth regulators (PGRs), for higher yields, is gaining momentum. PGR-induced higher yields are due to altered photosynthate distributive patterns within the plant and as such do not require any additional agricultural inputs.

Plant growth and development is a complex process and two classes of mechanisms that influence and regulate the developmental pattern of a plant have been recognized. The first is a system of endogenous chemical messengers, called hormones, that co-ordinate the development of individual organs as well as the plant as whole. The second class of mechanism comprise of extrinsic factors, such as light, water, temperature and gravity, which originate outside the organism and convey the information about the environment. Thus, the final pattern of development and behavior of each individual plant is the result of complex interplay between genetic, hormonal and environmental factors.

Application of growth retardants reduce yield due to altered source sink relationship, reduced plant height and canopy size. But, optimum concentration and single application of retardants at appropriate stage increase the yield by optimizing source sink relationship. Multiple application and higher concentration reduce yield by altering enzyme/hormonal concentration through protein synthesis and secondary messengers. Changes in plant growth and development are brought about by changing enzyme pattern in the plant cells.

With this in view, an experiment was conducted at Punjab Agricultural University, Ludhiana during *kharif* 2010-11 and 2011-12 to screen 25 pigeonpea genotypes on the basis of flowering and pod set and to study the effect of foliar spray of plant growth regulators on various morpho-physiological traits, biochemical constituents, yield and yield components of pigeonpea genotypes.

4.1 Experiment I

Physiological evaluation of twenty five pigeonpea (*Cajanus cajan* L.) genotypes for flowering and pod set.

4.1.1 Morpho-physiological parameters

4.1.1.1 Plant height

Plant height in pigeonpea is affected by maturity duration, photoperiod and environment. Pigeonpea genotypes were generally tall, probably due to the influence of exposure to long-day conditions (Fig. 1).

In early-duration group, genotype AL 1758 attained maximum plant height (95 cm) and minimum was recorded in AL 1756 (80.5 cm) at vegetative stage. At flowering stage, a drastic increase in plant height was observed in AL 1758 (210.5 cm) followed by AL 1757 (208 cm) and lowest in AL 1811 (185.25 cm). At post-flowering stage, genotype AL 1758 showed maximum plant height (247 cm) and lowest was observed in AL 1817 (218.5 cm).

In mid-duration group, the maximum plant height was observed in AL 1770 (106.5 cm) followed by AL 1593 (103 cm) and minimum was recorded in AL 1778 (76 cm) at vegetative stage. At flowering stage, significantly maximum plant height was registered in AL 1702 (260.80 cm) followed by AL 1578 (260.75cm) and minimum in AL 1794 (218.5 cm). At post-flowering stage maximum plant height was observed in AL 1770 (273.5 cm) followed by AL 1702 (266.5 cm). At this stage, minimum plant height was observed in genotype AL 1794 (244.75 cm).

In late-duration group, maximum plant height was observed in genotype AL 1792 (92.25 cm) at vegetative stage, while at flowering and post-flowering stage genotype AL 1721 recorded maximum (239.25 cm and 263.25 cm respectively) plant height.

Among checks, variety AL 201 recorded maximum plant height both at vegetative (96.5 cm) and flowering (232.5 cm) stage while at post-flowering stage, variety P 992 recorded maximum plant height (261.25 cm).

The taller plants observed in mid and long-duration genotypes than early-duration genotypes in this study are in concordance with the findings of Egbe (2005), which stated that short duration genotypes of pigeonpea had shorter plants than medium and long duration genotypes in both sole and intercropping systems. Long and mid duration genotypes are generally tall because of their prolonged vegetative phase, while short duration genotypes are comparatively short in stature due to their short vegetative phase (Egbe and Vange 2008).

4.1.1.2 Number of branches/plant

Number of branches/plant varied significantly among early, mid and late-duration genotypes (Fig. 2). Late-duration group produced maximum number of branches than early, mid-duration and local check group, which were 19.54 and 12.64 % higher than local check at flowering and post-flowering stage, respectively.

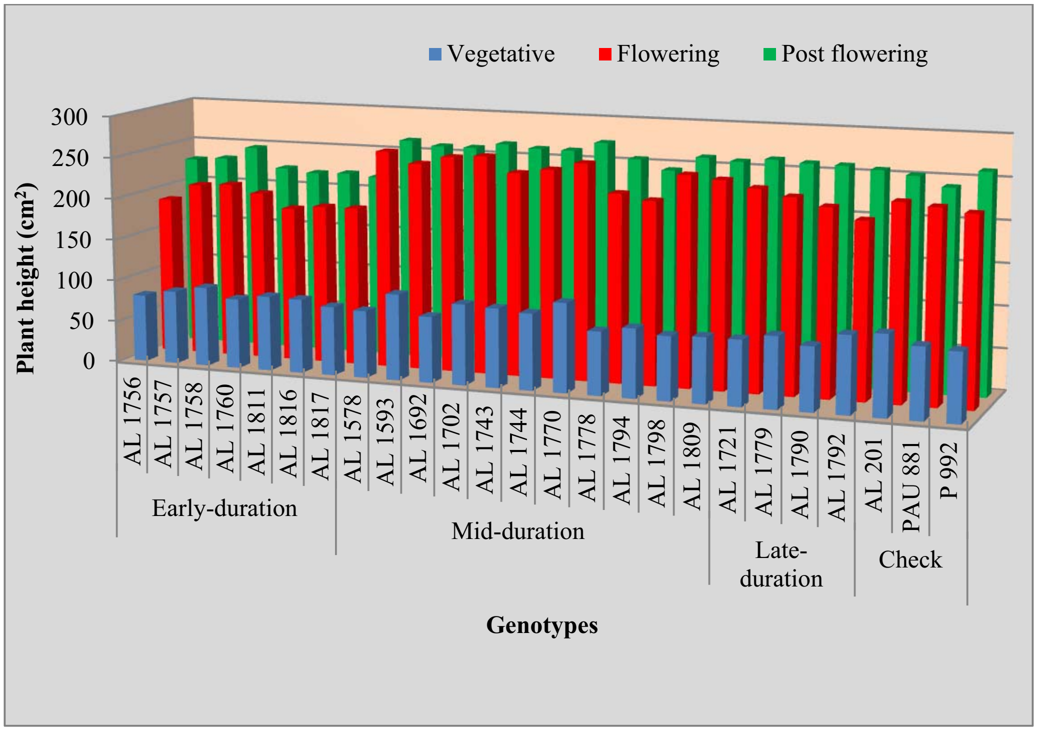


Fig. 1: Plant height in pigeonpea genotypes at different growth stages

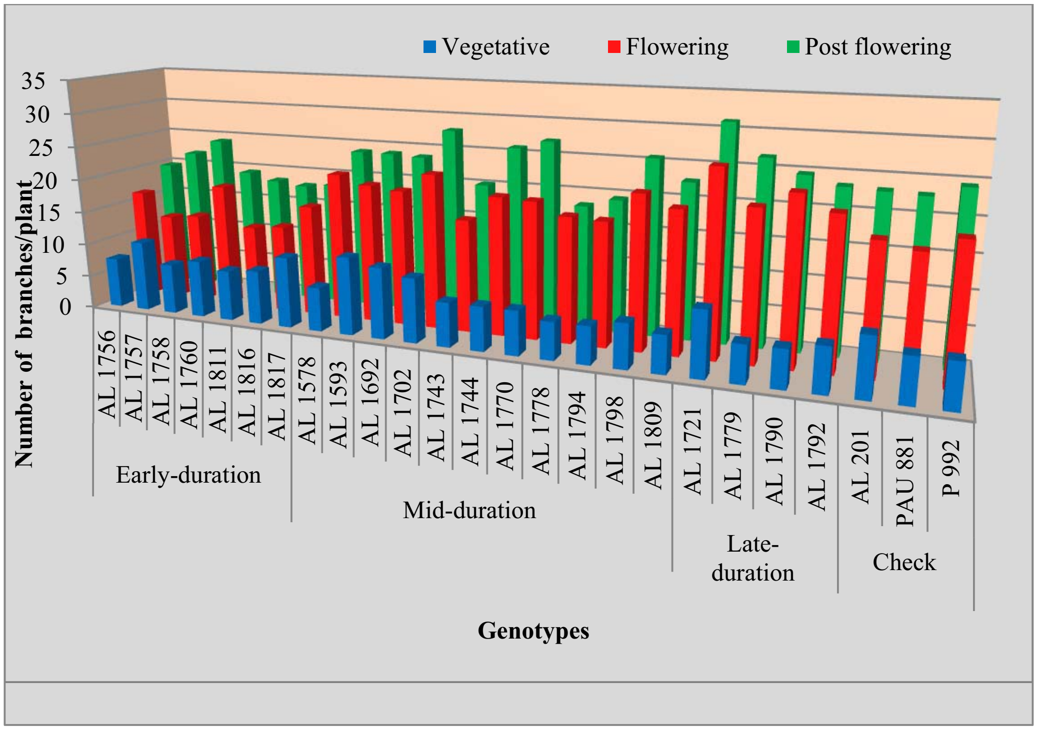


Fig. 2: Number of branches/plant in pigeonpea genotypes at different growth stages

In early-duration group, genotype AL 1757 and AL 1817 produced maximum (10.5) number of branches followed by genotype AL 1760 (8.5) at vegetative stage. At flowering stage, genotype AL 1760 produced maximum (18.5) number of branches while minimum was recorded in genotype AL 1811 (12.5). Also at post-flowering stage, genotype AL 1758 produced maximum (24.25) number of branches followed by AL 1757 (22) and minimum was recorded in AL 1816 (18.0).

Among mid-duration group, maximum number of branches/plant was recorded in AL 1593 (11.5) followed by AL 1692 (10.5) at vegetative stage. At flowering stage, genotype AL 1702 recorded highest (22.75) number of branches/plant followed by AL 1798 (22.25) and minimum number of branches was recorded in AL 1743 (16.5). At post-flowering stage, minimum number of branches was recorded in genotype AL 1778 (18.25) and maximum was recorded in AL 1770 and AL 1744 (28).

Late-duration group showed maximum number of branches both at flowering and post-flowering stages. Genotype AL 1721 produced maximum number of branches/plant at all the growth stages among its group.

Among checks, AL 201 produced maximum number of branches at vegetative (8.5) stage but at flowering and post-flowering stage, genotype P 992 produced maximum (19.5 and 24.5, respectively) number of branches/plant. Deshmukh *et al* (2009) also reported variation in number of branches/plant among different genotypes of pigeonpea. Similar results were obtained by Patel *et al* (2009).

4.1.1.3 Growth efficiency

The assessment of yield variation in terms of growth and development is very complex, since it involves the effect of external factors on all the physiological processes in plants. It is well established that the infrastructure of the plant is decided by the growth parameters such as relative growth rate (RGR), crop growth rate (CGR), leaf area index (LAI) and specific leaf weight (SLW).

i) Relative growth rate (RGR)

RGR represents the increase in dry weight per unit of dry weight already present per unit time (Fig. 3). RGR was calculated at two stages: stage I (vegetative to flowering) and stage II (flowering to maturity). It was found that RGR declined with advancement in crop growth. In early-duration group, highest value of RGR was recorded in AL 1758 (0.122 g g^{-1} dry wt. day^{-1}) followed by AL 1757 (0.095 g g^{-1} dry wt. day^{-1}) and lowest value of RGR was recorded in AL 1817 (0.065 g g^{-1} dry wt. day^{-1}) at stage I. In mid-duration group, at stage I, maximum RGR value was recorded in AL 1744 (0.119 g g^{-1} dry wt. day^{-1}) followed by AL 1593 (0.11 g g^{-1} dry wt. day^{-1}) and minimum was recorded AL 1798 (0.078 g g^{-1} dry wt. day^{-1}). At stage II, maximum RGR values were recorded in AL 1798 (0.036 g g^{-1} dry wt. day^{-1}) followed by AL 1794 (0.033). Genotypes AL 1692, AL 1702 and AL 1770 differed non-significantly from each other. Minimum RGR was recorded in AL 1593 (0.020 g g^{-1} dry wt. day^{-1}).

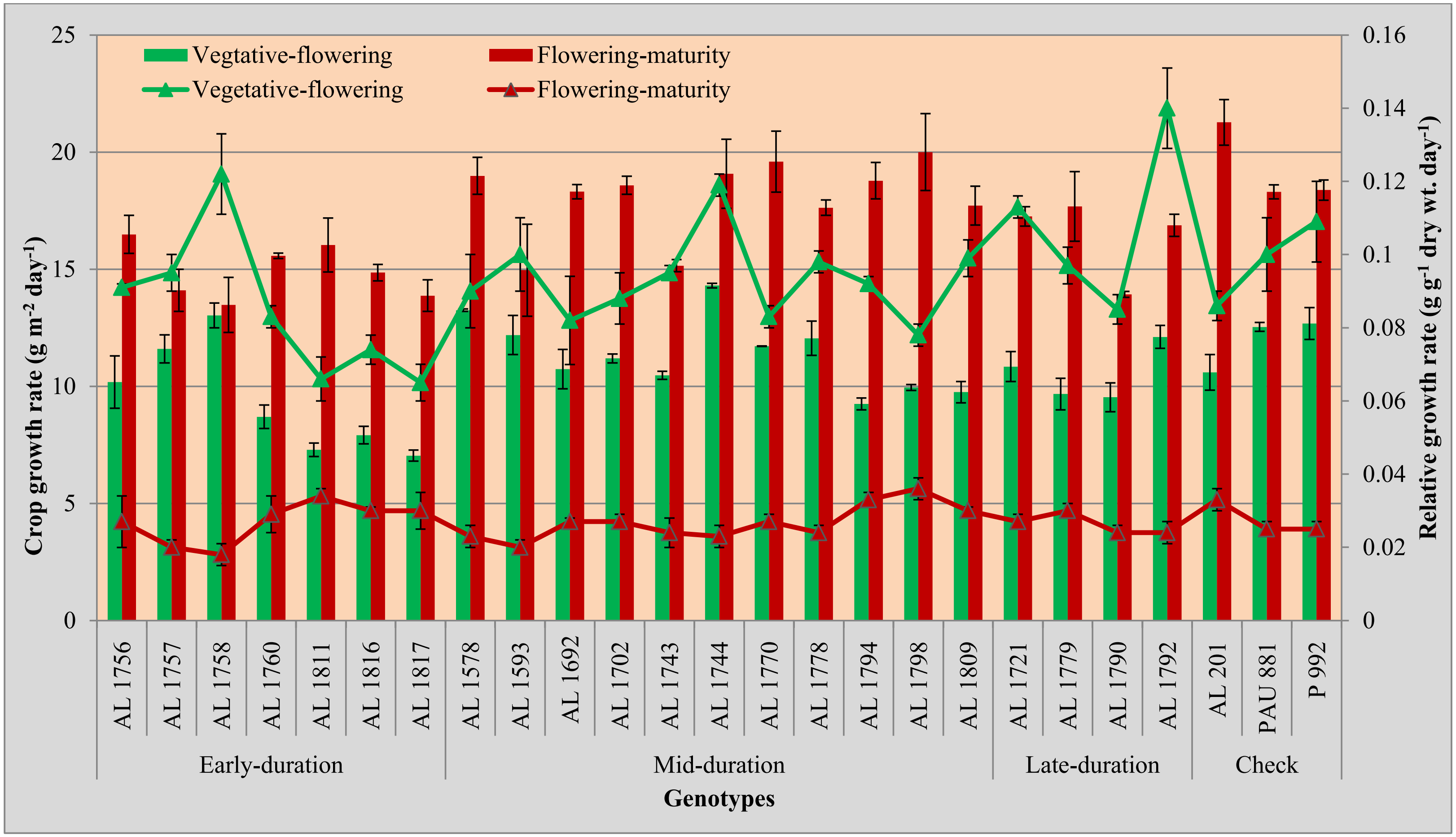


Fig. 3: Relative growth rate (RGR) and Crop growth rate (CGR) in pigeonpea genotypes

Among the late-duration group, AL 1792 recorded maximum (0.140 g g^{-1} dry wt. day^{-1}) RGR followed by AL 1721 (0.113 g g^{-1} dry wt. day^{-1}) and minimum value of RGR was recorded in AL 1790 (0.085 g g^{-1} dry wt. day^{-1}) at stage I. At stage II maximum RGR was recorded in AL 1779 (0.030 g g^{-1} dry wt. day^{-1}).

In check genotypes, maximum RGR was noticed in PAU 881 (0.119 g g^{-1} dry wt. day^{-1}) at stage I while at stage II, maximum RGR was recorded in AL 201 (0.033 g g^{-1} dry wt. day^{-1}).

The decline in RGR with the advancement of crop growth could be due to decline in the rate of dry matter production (Jirali 2001). Mondal *et al* (2012) reported that high yielding varieties should possess high RGR and NAR at vegetative stage which would result in superior yield components. Sharma *et al* (2010) reported significant positive correlation of CGR and RGR with seed yield.

ii) Crop growth rate (CGR)

CGR is a useful growth parameter for estimating the production efficiency of crop stand and it enables to make comparisons between the aspects of study. It was found that CGR increased with increase in crop duration (Fig.3). Among various groups, at stage I, check followed by mid- duration group showed maximum CGR (11.94 and 11.35 g m^{-2} day^{-1} respectively). Similarly at stage II, check genotypes (19.32 g m^{-2} day^{-1}) followed by mid duration group showed maximum (18.07 g m^{-2} day^{-1}) CGR and minimum (14.91 g m^{-2} day^{-1}) was recorded in early duration group.

Among early duration group, AL 1758 recorded maximum (13.03 g m^{-2} day^{-1}) CGR followed by AL 1757 (11.6 g m^{-2} day^{-1}) at stage I while at stage II, maximum CGR was recorded in AL 1756 (16.49 g m^{-2} day^{-1}) followed by AL 1811 (16.04 g m^{-2} day^{-1}) and minimum in AL 1758 (13.47 g m^{-2} day^{-1}).

In mid duration group, at stage I maximum CGR was recorded in AL 1744 (14.30 g m^{-2} day^{-1}) followed by AL 1578 (13.25 g m^{-2} day^{-1}), while at stage II, maximum CGR was recorded in AL 1798 (20.00 g m^{-2} day^{-1}) and minimum value of CGR was shown by AL 1593 (14.96 g m^{-2} day^{-1}).

Among long duration group, genotype AL 1792 showed maximum (12.11 g m^{-2} day^{-1}) CGR and minimum was recorded in AL 1790 (9.53 g m^{-2} day^{-1}) at stage I while at stage II, maximum CGR was shown by AL 1779 (17.68 g m^{-2} day^{-1}) and minimum was recorded in AL 1790 (13.93 g m^{-2} day^{-1}). Among local checks, AL 201 showed maximum (21.27) CGR and minimum was recorded in PAU 881 (18.30 g m^{-2} day^{-1}) at stage II.

Ullah (2006) reported that CGR depends on LAI and NAR, the latter depending on light intercepting efficiency and photosynthetic efficiency of a leaf. Increased CGR and NAR indicate the optimum physiological growth of a crop that produces maximum yield. Crop productivity is mainly determined by crop growth rate (CGR) which depends on leaf area index (LAI) and the rate of photosynthesis. The CGR was found to be less during early vegetative stage but increased with the advancement of growth in greengram (Kalubarme and Pandey 1979).

4.1.1.4 Leaf characteristics

i) Leaf area

Leaf area gives a good idea of the photosynthetic capacity of the plant. In the present study, leaf area increased drastically upto flowering stage and decreased thereafter (Fig 4).

In early duration group, maximum leaf area was recorded in genotype AL 1757 both at vegetative (2203.65 cm^2) and flowering (5155.06 cm^2) stage. Minimum leaf area at flowering stage was noticed in AL 1817 (3099.08 cm^2). Among mid duration group, genotype AL 1770 produced maximum (2501.50 cm^2) followed by AL 1578 (2489.87 cm^2) and minimum was recorded in AL 1809 (2102.3 cm^2) at vegetative stage. At flowering stage drastic increase in leaf area was observed in genotype AL 1744 (5108.36 cm^2) and minimum was noticed in AL 1743 (3706.8 cm^2). At post-flowering stage, genotype AL 1744 attained maximum (2492.26 cm^2) and minimum was attained by AL 1743 (2250.26 cm^2).

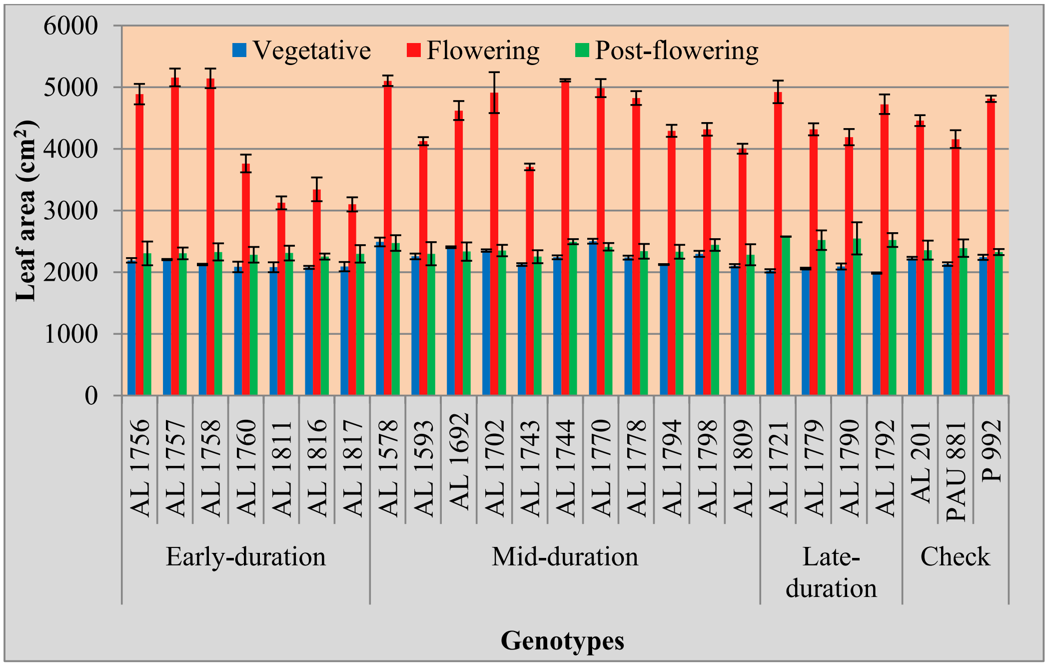


Fig.4 Leaf area in pigeonpea genotypes at different growth stages

Among late duration group, maximum leaf area was observed in genotype AL 1721, both at flowering (4922.18 cm^2) and post-flowering (2576.48 cm^2) stage. In local checks, genotype P 992 had maximum leaf area both at vegetative (2240.3 cm^2) and flowering (4810.5 cm^2) stage followed by AL 201 (2223.26 and 4455.69 cm^2, respectively).

ii) Leaf area index

Leaf area index (LAI) is a morphological trait that is used to predict photosynthetic primary production and studied as a reference tool for crop growth. LAI first increased from vegetative to flowering stage and then decreased with further advancing stage due to leaf abscission which resulted in lower number of leaves, reduced leaf area of plant and ultimately decreased LAI (Table 1).

Table 1: Leaf area index (LAI) in pigeonpea genotypes at different growth stages

Genotypes	**Stages**		
	Vegetative stage	**Flowering stage**	**Post-flowering stage**
Early-duration			
AL 1756	1.20±0.10	3.27±0.18	2.20±0.80
AL 1757	2.37±0.09	3.80±0.33	2.21±0.15
AL 1758	2.47±0.12	3.73±0.08	2.41±0.05
AL 1760	1.90±0.03	3.10±0.09	2.21±0.21
AL 1811	2.10±0.40	2.80±0.20	2.11±0.01
AL 1816	1.40±0.10	2.90±0.10	2.20±0.20
AL 1817	1.50±0.06	2.77±0.07	2.11±0.21
Mean	**1.85±0.13**	**3.19±0.15**	**2.21±0.23**
Mid-duration			
AL 1578	1.87±0.02	4.40±0.08	2.45±0.04
AL 1593	1.40±0.10	3.20±0.10	2.21±0.09
AL 1692	1.40±0.17	3.30±0.20	2.21±0.21
AL 1702	2.50±0.05	3.80±0.14	2.25±0.05
AL 1743	1.73±0.08	3.10±0.15	2.10±0.30
AL 1744	2.40±0.10	4.40±0.09	2.50±0.10
AL 1770	2.13±0.32	4.20±0.09	2.31±0.04
AL 1778	1.20±0.10	3.40±0.05	2.25±0.11
AL 1794	1.23±0.01	3.30±0.08	2.21±0.30
AL 1798	1.96±0.02	3.2±0.39	2.30±0.10
AL 1809	1.90±0.01	3.16±0.16	2.15±0.27
Mean	**1.79±0.09**	**3.59±0.14**	**2.27±0.15**
Late-duration			
AL 1721	1.40±0.09	3.87±0.28	2.49±0.10
AL 1779	2.10±0.03	3.77±0.07	2.41±0.07
AL 1790	2.40±0.19	3.20±0.24	2.40±0.03
AL 1792	1.70±0.08	3.73±0.14	2.61±0.07
Mean	**1.90±0.17**	**3.59±0.18**	**2.48±0.07**
Check			
AL 201	2.43±0.16	3.50±0.02	2.26±0.07
PAU 881	1.80±0.07	3.40±0.08	2.11±0.04
P 992	2.01±0.29	3.70±0.12	2.25±0.04
Mean	**2.09±0.17**	**3.53±0.07**	**2.21±0.05**
CD (5%)	**0.29**	**0.24**	**0.07**

In early duration group AL 1758 recorded maximum LAI (2.47) followed by AL 1757 (2.37) and lowest value of LAI was found in AL 1756 (1.2) at vegetative stage. However, at flowering stage, maximum LAI was found in AL 1757 (3.8) followed by AL 1758 (3.73) and lowest value of LAI was attained by AL 1817 (2.77). At post-flowering stage, all genotypes showed decrease in LAI due to senescence of matured leaves. Maximum LAI was observed in AL 1758 (2.41) followed by AL 1760 and AL 1757 (2.21) at this stage.

Among mid duration group, AL 1702 showed maximum (2.5) LAI followed by AL 1744 (2.4). Minimum LAI was recorded in AL 1778 (1.2) at vegetative stage. LAI increased at flowering stage in all the genotypes. Maximum LAI was recorded in genotype AL 1744 and it was at par with AL 1578 (4.4), while minimum LAI was observed in AL 1743 (3.1) at this stage. At post flowering stage, drastic decline in LAI was observed in all genotypes. AL 1744 had maximum (2.50) LAI and minimum was recorded in AL 1743 (2.1).

In late-duration group, genotype AL 1790 showed highest LAI (2.40) at vegetative stage while at flowering stage, genotype AL 1721 (3.87) followed by AL 1779 (3.77) showed maximum LAI. At post-flowering stage, a decline in LAI was recorded in all the genotypes. Maximum LAI was observed in AL 1792 (2.61) at this stage and minimum was recorded in AL 1790 (2.40). Among local checks genotype P 992 had maximum (3.70) LAI at flowering stage. At post-flowering stage genotype P 992 and AL 201 differed non-significantly from each other.

Malek *et al* (2012) observed that high yielding genotypes always showed superiority in total dry matter production (TDM) and LAI as compared to low yielding ones at most of the growth stages in soybean. This indicates that LAI and TDM are the most important parameters for increasing seed yield. Tesfaye *et al* (2006) reported that attainment of high LAI reduces soil water evaporation, intercepts and converts radiation into dry matter efficiently and partitioning of the dry matter to the seed which is the major requirement for high seed yield.

iii) Specific leaf weight (SLW)

The specific leaf weight indicates leaf thickness, which is due to compactness and stacking of mesophyll cells. Since pigeonpea is a C_3 plant, the photosynthetic efficiency per unit area is low and the increase in leaf thickness could probably be due to enhanced photosynthetic efficiency and more stacking of mesophyll cells.

In the present study, the SLW increased from vegetative stage till harvest. Mid duration group and local checks had maximum SLW at all the stages than early and late duration groups (Fig. 5). Among early duration group, genotype AL 1757 had maximum SLW at vegetative (4.18 mg cm^{-2}), flowering (6.21 mg cm^{-2}) and post flowering (6.6 mg cm^{-2}) stages. Minimum SLW was noticed in genotype AL 1817 at all stages. In mid duration group, AL 1578 recorded maximum SLW (4.58 mg cm^{-2}) and minimum was observed in AL 1809

(3.41 mg cm^{-2}) at vegetative stage. At flowering and post flowering stage maximum SLW was attained by AL 1744 (6.3, 7.33 mg cm^{-2} respectively) and minimum was recorded in AL 1743 (5.43 mg cm^{-2} at flowering stage) and AL 1809 (6.574 mg cm^{-2} at post-flowering stage).

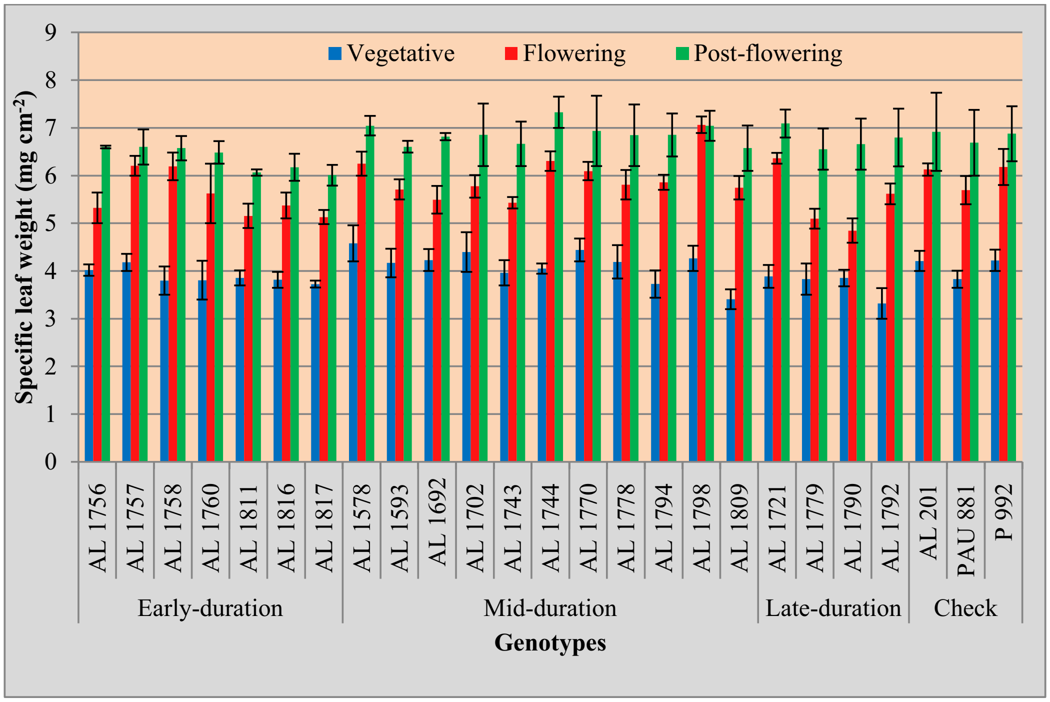


Fig. 5 Specific leaf weight (SLW) in pigeonpea genotypes at different growth stages

In late duration group, AL 1721 attained maximum SLW at all the growth stages (3.89, 6.36, 7.09 mg cm^{-2} respectively). Among local checks, P 992 had maximum SLW at vegetative (4.22 mg cm^{-2}) and flowering (6.18 mg cm^{-2}) stages while at post flowering stage AL 201 had maximum (6.92 mg cm^{-2}) SLW. Early and late duration group had 7 and 6 % less SLW than local check genotypes. Bhatia *et al* (1996) reported that SLW increases with the age of crop due to increased sink demand in soybean plants. In common bean, higher yielding cultivars had thicker leaves due to increased mesophyll volume, which led to greater relative growth rates and leaf assimilation rates (White and Montes 2005).

iv) Specific leaf area (SLA)

The inverse of SLW is SLA. In general SLA decreased as the age of the crop advanced. SLA was maximum at vegetative stage and decreased towards maturity (Table 2). Among early- duration group, maximum SLA was observed in AL 1817 at all growth stages (0.269, 0.195 and 0.166 cm^{2} mg^{-1}). In mid duration group, highest SLA was attained by AL 1809 (0.29 cm^{2} mg^{-1}) at vegetative stage. At flowering stage AL 1743 had maximum (0.184 cm^{2} mg^{-1}) SLA while at post-flowering stage AL 1809 had maximum (0.152 mg cm^{-2}) SLA.

In late duration group, AL 1792 attained maximum (0.301 cm^{2} mg^{-1}) SLA at vegetative stage, while at flowering stage AL 1790 had maximum (0.206 cm^{2} mg^{-1}) SLA. Among local check genotypes, PAU881 attained highest SLA at all the growth stages (0.261, 0.176 and 0.149 cm^2 mg^{-1} at vegetative, flowering and post-flowering stages respectively), which was 10, 8 and 4 % higher than local check AL 201 at all growth stages respectively.

Table 2: Specific leaf area (SLA) in pigeonpea genotypes at different growth stages

Genotypes	**Stages**		
	Vegetative stage	**Flowering stage**	**Post-flowering stage**
Early-duration			
AL 1756	0.249±0.004	0.188±0.007	0.152±0.003
AL 1757	0.239±0.005	0.161±0.007	0.152±0.005
AL 1758	0.263±0.001	0.161±0.002	0.152±0.011
AL 1760	0.263±0.018	0.178±0.017	0.154±0.003
AL 1811	0.259±0.004	0.194±0.013	0.165±0.005
AL 1816	0.262±0.003	0.186±0.008	0.162±0.005
AL 1817	0.269±0.009	0.195±0.011	0.166±0.007
Mean	**0.258±0.006**	**0.180±0.009**	**0.158±0.005**
Mid-duration			
AL 1578	0.218±0.018	0.160±0.040	0.142±0.002
AL 1593	0.240±0.02	0.175±0.004	0.151±0.001
AL 1692	0.236±0.006	0.182±0.003	0.147±0.005
AL 1702	0.228±0.018	0.173±0.003	0.146±0.013
AL 1743	0.252±0.020	0.184±0.003	0.150±0.020
AL 1744	0.247±0.006	0.159±0.005	0.136±0.006
AL 1770	0.225±0.018	0.164±0.005	0.144±0.005
AL 1778	0.239±0.020	0.172±0.007	0.146±0.005
AL 1794	0.268±0.006	0.171±0.006	0.146±0.016
AL 1798	0.235±0.018	0.142±0.003	0.142±0.007
AL 1809	0.294±0.002	0.174±0.006	0.152±0.002
Mean	**0.244±0.016**	**0.169±0.008**	**0.146±0.007**
Late-duration			
AL 1721	0.257±0.033	0.157±0.008	0.141±0.011
AL 1779	0.261±0.021	0.196±0.001	0.153±0.013
AL 1790	0.260±0.010	0.206±0.006	0.150±0.020
AL 1792	0.301±0.021	0.178±0.008	0.147±0.013
Mean	**0.270±0.021**	**0.184±0.006**	**0.148±0.014**
Check			
AL 201	0.238±0.010	0.163±0.005	0.145±0.025
PAU 881	0.261±0.259	0.176±0.005	0.149±0.010
P 992	0.237±0.017	0.162±0.004	0.145±0.002
Mean	**0.245±0.095**	**0.167±0.005**	**0.146±0.012**
CD (5%)	0.01	0.04	0.012

4.1.2 Phenological traits and number of flowers and pods

i) Days to 50% flowering and maturity

Number of days to 50 % flowering is expressed in terms of time taken for 50% flowering from days after sowing (DAS). Significant differences were observed among all groups (early, mid, late-duration and local checks) for days taken to achieve 50% flowering (Fig. 6). Among these, early duration group genotypes achieved 50% flowering earlier (86 DAS) followed by local checks (92 DAS) and mid-duration (95 DAS). Maximum days to 50% flowering were taken by late-duration group (107 DAS).

In early-duration group, genotype AL 1756 recorded early flower initiation (80 DAS) followed by AL 1757 (81 DAS), while maximum number of days were taken by AL 1817 (89 DAS). Among mid-duration group, genotypes AL 1743 and AL 1778 took minimum (93 DAS) days to flower and maximum days were taken by AL 1702 (96 DAS). In late-duration group, genotype AL 1792 took minimum days to flower (101 DAS) and maximum days were taken by AL 1790 (115 DAS). Similarly the number of days taken to reach maturity was lowered in early duration group and higher in late-duration group (Fig 6).

ii) Number of flowers produced

Wide variation in number of flowers/plant was observed in all genotypes (Fig. 7). In early duration group maximum number of flowers/plant was observed in AL 1817 (983.77) followed by AL 1756 (980.8) and minimum in AL 1816 (841). In mid duration group, genotype AL 1702 produced maximum (982.22) number of flowers/plant followed by genotype AL 1778 (960) and AL 1794 (959.54) and minimum in AL 1744 (801).

Among long duration genotypes, AL1790 produced maximum (898.88) number of flowers/plant and in check genotypes , PAU 881 produced maximum (907.65) number of flowers/plant.

iii) Number of pods/plant

Number of pods produced/plant were less than number of flowers/plant due to abscission of flowers which is a major limitation in legumes (Fig. 7). On an average, mid duration genotypes produced maximum number of pods as compared to early, late duration and check genotypes, which was 28, 22 and 14 % higher than respective groups.

Among early duration group, AL 1756 produced maximum number (169.17) of pods. In mid and late duration group, genotype AL 1744 (218.8) and AL 1721 (160.75) produced maximum number of pods/plant respectively. Among check genotypes, AL 201 produced maximum (169.60) number of pods/plant.

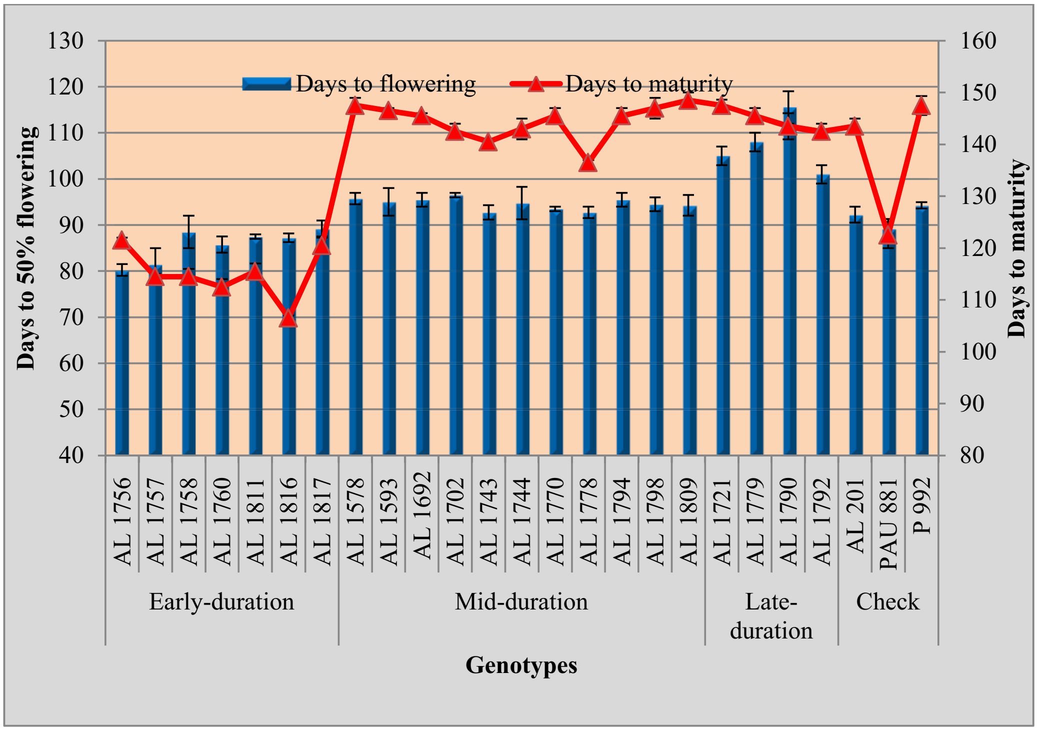


Fig. 6: Days to 50% flowering and maturity in pigeonpea genotypes

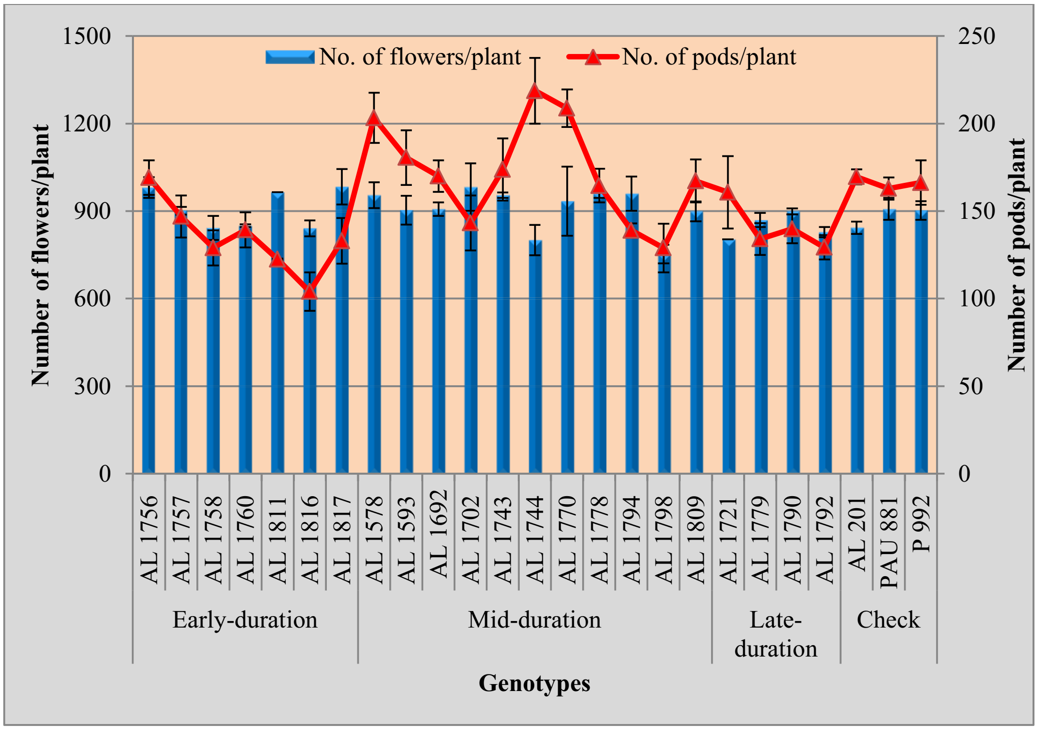


Fig. 7: Number of flowers and pods/plant in pigeonpea genotypes

4.1.3 Partitioning coefficient

Dry matter partitioning coefficient of leaves, shoots, flowers and pods were recorded at different growth stages (Table 3 ,4,5 and 6).

At vegetative stage, leaf dry matter was highest in all genotypes than shoot dry matter, therefore, partitioning coefficient was higher in leaf as compared to shoot (Table 3). Among early duration group, genotype AL 1757 had maximum (15.27g) total dry matter (TDM) followed by AL 1756 (14.02g). Leaf partitioning coefficient was maximum in AL 1756 (62.77) followed by AL 1758 (60.56) and minimum was recorded in AL 1817 (57.54). Shoot partitioning coefficient followed a reverse trend as that of leaf partitioning coefficient (Table 3). Genotype AL 1817 had maximum (42.45) shoot partitioning coefficient while minimum was recorded in AL 1756 (37.23). In mid duration group, leaf partitioning coefficient was maximum in AL 1702 (64.74) followed by AL 1770 (62.63) and minimum in AL 1809 (58.31). Shoot partitioning coefficient was maximum in AL 1809 (41.69) followed by AL 1744 (39.65) and AL 1778 (38.84). In late duration group, maximum TDM was observed in genotype AL 1790 (13.96 g). Leaf partitioning coefficient was maximum in AL 1721 (65.77). Genotype AL 1790 had maximum shoot partitioning coefficient (42.33%) followed by AL 1792 (39.13%) and minimum was calculated in AL 1721 (34.23%). Among local checks, P 992 had maximum leaf partitioning coefficient (64.97 %).

Total dry matter (TDM) increased from vegetative to flowering stage. More dry matter got accumulated in shoots and leaves to support the reproductive parts. At flowering stage, among early duration group, AL 1758 had maximum TDM (94.73g) (Table 4). Maximum shoot partitioning coefficient was observed in AL 1811 (71.24 %) and minimum in AL 1757 (61.71 %), leaf partitioning coefficient was maximum in AL 1757 (36.46%) and minimum in AL 1811 (27.15 %). Genotype AL 1757 was more efficient in partitioning maximum dry matter towards flowers (1.82 %) while minimum partitioning towards flowers was observed in AL 1817 (1.55). Among mid-duration group, genotype AL 1744 showed maximum TDM (104.42 g). Maximum shoot partitioning was observed in AL 1743 (73.25%), which showed minimum leaf partitioning coefficient (25.43%). Flower partitioning coefficient was maximum in AL 1798 (2.18%) followed by AL 1744 (1.92 %) and minimum in AL 1743 (1.31). In late duration group, AL 1792 recorded maximum (86.48g) TDM. Flower partitioning coefficient was maximum in AL 1721 (1.98%). Among local checks, minimum TDM (81.53) was recorded in AL 201, as compared to other checks but it showed maximum (1.94%) flower partitioning coefficient.

At post-flowering stage, due to formation of pods, partitioning of dry matter gets accelerated towards reproductive organs as compared to leaves (Table 5). In early duration group, AL 1756 showed maximum (143.84 g) TDM followed by genotype AL 1758 (141 g) and AL 1757 (137.67 g). Shoot partitioning coefficient was maximum in AL 1816 (77.79%)

Table 3: Dry matter (DM) and partitioning coefficient at vegetative stage

Genotypes	**DM and partitioning**				
	Leaf dry matter	**Shoot dry matter**	**Total dry matter**	**Leaf partitioning coefficient**	**Shoot partitioning coefficient**
Early-duration					
AL 1756	8.80±0.10	5.22±0.22	14.02±0.32	62.77	37.23
AL 1757	9.21±1.91	6.06±0.21	15.27±2.12	60.31	39.69
AL 1758	8.06±0.14	5.25±0.25	13.31±0.39	60.56	39.44
AL 1760	7.94±0.44	5.21±0.23	13.15±0.67	60.38	39.62
AL 1811	8.02±0.37	5.74±0.74	13.76±1.11	58.28	41.72
AL 1816	7.92±0.33	5.52±0.40	13.44±0.73	58.93	41.07
AL 1817	7.78±0.10	5.74±0.10	13.52±0.20	57.54	42.45
Mean	**8.25±0.25**	**5.53±0.53**	**13.78±0.78**	**59.82**	**40.18**
Mid-duration					
AL 1578	11.4±0.40	7.03±0.19	18.43±0.59	61.86	38.14
AL 1593	9.40±0.50	5.85±0.49	15.25±0.99	61.64	38.36
AL 1692	10.16±1.20	6.20±0.20	16.36±1.40	62.10	37.9
AL 1702	10.32±0.24	5.62±0.39	15.94±0.63	64.74	35.26
AL 1743	8.41±1.05	5.32±0.22	13.73±1.27	61.25	38.75
AL 1744	9.07±0.71	5.96±0.15	15.03±0.86	60.35	39.65
AL 1770	11.11±1.19	6.63±0.40	17.74±1.59	62.63	37.37
AL 1778	9.37±1.32	5.95±0.21	15.32±1.53	61.16	38.84
AL 1794	7.91±0.45	4.71±0.12	12.62±0.57	62.68	37.32
AL 1798	9.79±0.51	6.07±0.18	15.86±0.69	61.73	38.27
AL 1809	7.16±1.14	5.12±0.12	12.28±1.26	58.31	41.69
Mean	**9.46±0.43**	**5.86±0.31**	**15.32±0.74**	**61.68**	**38.32**
Late-duration					
AL 1721	7.86±0.44	4.09±0.11	11.95±0.55	65.77	34.23
AL 1779	7.87±0.89	4.57±0.27	12.44±1.16	63.26	36.74
AL 1790	8.05±0.75	5.91±0.45	13.96±1.20	57.67	42.33
AL 1792	6.58±0.38	4.23±0.13	10.81±0.51	60.87	39.13
Mean	**7.59±0.21**	**4.7±0.09**	**12.29±0.30**	**61.89**	**38.11**
Check					
AL 201	9.36±0.03	5.97±0.74	15.33±0.77	61.06	38.94
PAU 881	8.15±0.80	5.00±0.19	13.15±0.99	61.98	38.02
P 992	9.46±1.10	5.10±0.40	14.56±1.50	64.97	35.03
Mean	**8.99±0.89**	**5.36±0.26**	**14.35±1.15**	**62.67**	**37.33**

Table 4: Dry matter (DM) and partitioning coefficient at flowering stage

Genotypes		DM and partitioning						
		Shoot dry matter	**Leaf dry matter**	**Flower dry matter**	**Total dry matter**	**Shoot partitioning**	**Leaf partitioning**	**Flower partitioning**
Early-duration	**AL 1756**	50.44±2.10	26.00±2.00	1.23±0.40	77.67±4.50	64.94	33.47	1.58
	AL 1757	54.16±3.93	32.00±1.70	1.60±0.11	87.76±5.74	61.71	36.46	1.82
	AL 1758	61.36±2.36	31.83±2.51	1.54±0.21	94.73±5.08	64.77	33.60	1.63
	AL 1760	45.27±2.02	21.15±1.79	1.10±0.10	67.52±3.91	67.05	31.32	1.63
	AL 1811	42.25±1.89	16.10±0.74	0.96±0.05	59.31±2.68	71.24	27.14	1.62
	AL 1816	44.06±1.70	17.95±1.65	0.90±0.00	62.91±3.35	70.04	28.53	1.43
	AL 1817	40.72±1.32	15.90±1.67	0.89±0.29	57.51±3.28	70.81	27.65	1.55
	Mean	**48.32±0.82**	**22.99±1.76**	**1.17±0.78**	**72.49±3.36**	**67.22**	**31.17**	**1.61**
Mid-duration	**AL 1578**	67.56±5.20	31.90±1.40	1.78±0.19	101.24±6.79	66.73	31.51	1.76
	AL 1593	66.48±2.20	23.54±1.44	1.39±0.32	91.41±3.96	72.73	25.75	1.52
	AL 1692	56.65±0.00	25.36±1.71	1.42±0.26	83.43±1.97	67.90	30.40	1.70
	AL 1702	55.99±5.63	28.36±1.00	1.52±0.27	85.87±6.90	65.20	33.03	1.77
	AL 1743	57.98±3.62	20.13±1.98	1.04±1.01	79.15±6.61	73.25	25.43	1.31
	AL 1744	70.22±2.02	32.20±2.10	2.00±0.90	104.42±5.02	67.25	30.84	1.92
	AL 1770	58.88±3.51	30.35±1.99	1.70±0.13	90.93±5.63	64.75	33.38	1.87
	AL 1778	60.99±2.63	28.00±1.85	1.65±0.22	90.64±4.70	67.29	30.89	1.82
	AL 1794	44.08±1.73	25.14±1.00	1.24±0.60	70.46±3.33	62.56	35.68	1.76
	AL 1798	45.85±3.49	30.47±2.11	1.70±0.02	78.02±5.62	58.77	39.05	2.18
	AL 1809	48.93±3.93	22.98±2.85	1.28±0.52	73.19±7.30	66.85	31.40	1.75
	Mean	**57.60±2.60**	**27.13±1.97**	**1.52±0.25**	**86.25±4.82**	**66.66**	**31.58**	**1.76**
Late-duration	**AL 1721**	46.79±4.79	31.32±1.17	1.58±0.06	79.69±6.02	58.72	39.30	1.98
	AL 1779	49.56±4.56	22.00±1.82	1.32±0.27	72.88±6.65	68.00	30.19	1.81
	AL 1790	52.29±2.06	20.30±1.94	0.96±0.51	73.55±4.51	71.09	27.60	1.31
	AL 1792	58.54±7.18	26.52±2.04	1.42±0.28	86.48±9.50	67.69	30.67	1.64
	Mean	**51.80±2.24**	**25.04±0.94**	**1.32±0.48**	**78.15±3.66**	**66.38**	**31.94**	**1.69**
Check	**AL 201**	52.65±2.52	27.30±1.96	1.58±0.12	81.53±4.60	64.58	33.48	1.94
	PAU 881	66.29±1.93	23.67±1.37	1.54±0.24	91.5±3.54	72.45	25.87	1.68
	P 992	62.46±2.10	29.73±1.61	1.60±0.09	93.79±3.80	66.60	31.70	1.71
	Mean	**60.47±2.18**	**26.90±1.65**	**1.57±0.15**	**88.94±3.98**	**67.87**	**30.35**	**1.78**

Table 5: Dry matter (DM) and partitioning coefficient at Post-flowering stage

Genotypes		**DM and partitioning**						
		Shoot DM	**Leaf DM**	**Pods DM**	**Total DM**	**Shoot partitioning**	**Leaf partitioning**	**Pod partitioning**
Early-duration	**AL 1756**	104.75±3.85	15.20±0.60	23.89±4.76	143.84±9.21	72.82	10.57	16.61
	AL 1757	97.17±4.81	15.20±0.30	25.30±2.06	137.67±7.17	70.58	11.04	18.38
	AL 1758	101.93±2.93	15.30±0.64	23.77±3.41	141.00±6.98	72.29	10.85	16.86
	AL 1760	101.27±3.27	14.80±0.60	21.49±0.83	137.56±4.70	73.62	10.76	15.62
	AL 1811	102.80±3.44	14.00±1.00	17.20±2.10	134.00±6.54	76.72	10.45	12.84
	AL 1816	105.21±2.21	13.90±0.80	16.14±1.14	135.25±4.15	77.79	10.28	11.93
	AL 1817	106.42±5.19	13.80±0.82	17.10±1.10	137.32±7.11	77.50	10.05	12.45
	Mean	**102.79±3.67**	**14.60±0.68**	**20.70±2.20**	**138.09±6.55**	**74.47**	**10.57**	**14.96**
Mid-duration	**AL 1578**	127.15±11.15	17.41±0.41	29.00±3.70	173.56±15.26	73.26	10.03	16.71
	AL 1593	109.95±4.95	15.17±0.81	22.07±1.77	147.19±7.53	74.70	10.31	14.99
	AL 1692	111.30±8.91	15.90±0.49	23.91±2.68	151.11±12.08	73.65	10.52	15.82
	AL 1702	115.98±2.98	16.10±1.00	27.40±1.90	159.48±5.88	72.72	10.10	17.18
	AL 1743	100.52±3.16	15.00±1.02	18.88±1.68	134.40±5.86	74.79	11.16	14.05
	AL 1744	127.15±2.15	18.26±0.57	30.18±1.81	175.59±4.53	72.41	10.40	17.19
	AL 1770	127.91±6.69	16.71±1.06	27.60±6.24	172.22±13.99	74.27	9.70	16.03
	AL 1778	115.61±3.61	16.00±0.61	25.10±1.74	156.71±5.96	73.77	10.21	16.02
	AL 1794	124.18±7.82	15.96±1.66	23.00±2.11	163.14±11.59	76.12	9.78	14.10
	AL 1798	124.62±3.38	17.20±1.00	28.56±0.91	170.38±5.29	73.14	10.10	16.76
	AL 1809	109.68±9.32	15.00±0.90	22.00±1.64	146.68±11.86	74.78	10.23	15.00
	Mean	**117.64±5.83**	**16.25±0.87**	**25.25±2.38**	**159.13±9.07**	**73.97**	**10.23**	**15.80**
Late-duration	**AL 1721**	108.98±13.32	18.27±0.29	23.00±1.64	150.25±15.25	72.53	12.16	15.31
	AL 1779	104.61±5.39	16.50±1.14	21.45±2.09	142.56±8.62	73.38	11.57	15.05
	AL 1790	93.99±2.01	16.95±0.85	19.60±1.24	130.54±4.10	72.00	12.98	15.01
	AL 1792	115.74±6.82	17.13±0.59	22.45±2.12	155.32±9.53	74.52	11.03	14.45
	Mean	**105.83±6.88**	**17.21±0.72**	**21.63±1.77**	**144.67±9.37**	**73.11**	**11.94**	**14.96**
Check	**AL 201**	120.47±8.53	16.30±0.98	26.77±2.67	163.54±12.18	73.66	9.97	16.37
	PAU 881	121.45±5.94	15.98±1.38	21.89±2.02	159.32±9.34	76.23	10.03	13.74
	P 992	122.68±5.32	15.98±1.92	25.18±2.20	163.84±9.44	74.88	9.75	15.37
	Mean	**121.53±6.60**	**16.09±1.43**	**24.61±2.30**	**162.23±10.32**	**74.92**	**9.92**	**15.16**

Table 6: Dry matter (DM) and partitioning coefficient at maturity stage

Genotypes		DM and partitioning						
		Shoot DM	Leaf DM	Pod DM	Total DM	Shoot partitioning	Leaf partitioning	Pod partitioning
Early-duration	**AL 1756**	114.25±10.25	1.90±0.20	54.35±4.35	170.50±14.80	67.01	1.11	31.88
	AL 1757	108.20±6.20	2.00±0.30	56.92±7.92	167.12±14.42	64.74	1.20	34.06
	AL 1758	111.15±8.85	1.87±0.22	57.55±6.55	170.57±15.62	65.16	1.10	33.74
	AL 1760	104.99±11.01	1.85±0.07	48.36±2.36	155.20±13.44	67.65	1.19	31.16
	AL 1811	101.13±20.87	1.79±0.14	46.68±4.68	149.60±25.69	67.60	1.20	31.20
	AL 1816	99.37±1.37	1.82±0.02	45.35±5.35	146.54±6.74	67.81	1.24	30.95
	AL 1817	92.44±7.44	1.78±0.05	41.38±2.18	135.60±9.67	68.17	1.31	30.52
	Mean	**104.50±4.50**	**1.86±0.04**	**50.08±2.69**	**156.45±7.23**	**66.88**	**1.19**	**31.93**
Mid-duration	**AL 1578**	130.82±8.82	2.74±0.06	74.58±4.22	208.14±13.10	62.85	1.32	35.83
	AL 1593	115.60±4.40	2.10±0.10	57.93±3.57	175.63±8.07	65.82	1.20	32.98
	AL 1692	119.87±2.13	2.10±0.20	64.54±2.24	186.51±4.57	64.27	1.13	34.60
	AL 1702	120.70±2.30	2.40±0.14	67.39±2.25	190.49±4.69	63.36	1.26	35.38
	AL 1743	107.50±3.50	1.80±0.30	55.14±4.78	164.44±8.58	65.37	1.09	33.53
	AL 1744	132.75±4.25	2.80±0.30	76.25±3.89	211.80±8.44	62.68	1.32	36.00
	AL 1770	129.40±2.60	2.62±0.18	69.23±4.03	201.25±6.81	64.30	1.30	34.40
	AL 1778	120.20±2.80	2.40±0.11	67.28±4.89	189.88±7.80	63.30	1.26	35.43
	AL 1794	114.50±2.50	2.10±0.16	59.59±4.36	176.19±7.02	64.99	1.19	33.82
	AL 1798	130.30±5.70	2.65±0.07	71.00±2.00	203.95±7.77	63.89	1.30	34.81
	AL 1809	114.87±2.13	1.94±0.05	56.11±5.11	172.92±7.29	66.43	1.12	32.45
	Mean	**121.50±5.50**	**2.33±0.03**	**65.37±4.01**	**189.20±9.54**	**64.30**	**1.23**	**34.48**
Late-duration	**AL 1721**	114.50±3.50	2.30±0.02	60.02±1.66	176.82±5.18	64.76	1.30	33.94
	AL 1779	114.50±4.50	3.00±0.11	54.94±4.58	172.44±9.19	66.40	1.74	31.86
	AL 1790	104.45±8.55	2.50±0.20	45.00±3.77	151.95±12.52	68.74	1.65	29.62
	AL 1792	119.48±9.52	1.90±0.03	60.10±2.74	181.48±12.29	65.84	1.05	33.12
	Mean	**113.23±3.77**	**2.43±0.03**	**55.02±3.63**	**170.67±7.43**	**66.43**	**1.43**	**32.13**
Check	**AL 201**	130.40±7.04	1.86±0.06	69.00±3.61	201.26±10.71	64.79	0.92	34.28
	PAU 881	131.50±3.50	1.79±0.04	61.23±2.27	194.52±5.81	67.60	0.92	31.48
	P 992	130.94±2.06	2.00±0.03	64.34±1.98	197.28±4.07	66.37	1.01	32.61
	Mean	**130.95±2.61**	**1.88±0.08**	**64.86±2.88**	**197.69±5.57**	**66.26**	**0.95**	**32.79**

and minimum shoot partitioning coefficient was found in genotype AL 1757 (70.58%) as it partition maximum of dry matter towards pods (18.38%) and leaves (11.04%) followed by AL 1758 (16.86 and 10.85 respectively). Among mid duration group, genotype AL 1744 attained maximum (175.59 g) TDM followed by AL 1578 (173.56 g). Maximum shoot partitioning coefficient was observed in AL 1794 which alternatively showed minimum leaf (9.78%) and pods (14.10%) partitioning coefficient while maximum pods partitioning coefficient was observed in AL 1744 (17.19%) followed by AL 1702 (17.18%). Mid duration group showed maximum pod partitioning coefficient as compared to all other groups studied. In late duration group AL 1792 had maximum (155.32 g) TDM followed by AL 1721 (150.25 g). Maximum shoot partitioning was observed in AL 1792 (74.52%), while it showed minimum pods partitioning coefficient (14.45%) and leaf partitioning coefficient (11.03%). Maximum pod partitioning was observed in AL 1721 (15.31). Among local checks, P992 and AL 201 differed non-significantly from each other and showed 163.84 and 163.54 g TDM respectively. Shoot partitioning coefficient was maximum in PAU 881 (76.23%). Maximum pod partitioning was observed in AL 201 (16.37%).

At maturity stage, partitioning coefficient towards pods increased while in shoot and leaves decreased due to accumulation of more DM in pods as compared to post-flowering stage. In general mid duration group showed maximum pod partitioning coefficient (Table 6). Among early group, genotype AL 1758 showed maximum (170.57 g) while AL 1817 showed minimum (135.6 g) TDM. Genotype AL 1817 partitioned more dry matter towards shoots (68.17%) as compared to all other genotypes, therefore, DM partitioning towards pods got decreased. Maximum pod partitioning (34.06%) and minimum shoot (64.74%) and leaves (1.20%) partitioning was shown by AL 1757. In mid duration group, maximum TDM was accumulated in AL 1744 (211.80 g) followed by AL 1578 (208.14 g). Maximum pod partitioning was achieved by AL 1744 (36%) followed by AL 1578 (35.83%) and minimum in AL 1809 (32.45). In long duration group, maximum pod partitioning was noticed in AL 1721 (33.94%) and minimum in AL 1790 (29.62). In check genotypes, AL 201 showed maximum (34.28%) pod partitioning coefficient and minimum was calculated in PAU881 (31.48 %).

Tekale *et al* (2009) reported that the movement of photoassimilates from the site of synthesis in leaf tissues (source) to the site of net accumulation in different tissues (sink) is under the potential control of numerous factors. Regulation of net flow of photoassimilates is an integrated process. The concentration gradient and ability of sink to assimilates between the source and sink is the primary determinant of the current rate of transport and pattern of partitioning.

4.1.4 Yield parameters

i) Pod weight/plant

Among the various groups, mid duration group recorded highest pod weight (Fig. 8). In early duration group, AL 1758 produced maximum pod weight (57.55g) and lowest was noticed in AL 1817 (41.38g). In mid duration group, pod weight ranges from (55.14-76.25). Maximum pod weight was recorded in AL 1744 (76.25 g) followed by AL 1578 (74.58g). Among late duration group, AL 1721 and AL 1792 differed non-significantly, as these genotypes produced 60.02 and 60.1 g pod weight/plant, respectively. In local checks, AL 201 produced maximum pod weight (69 g) which was 13% higher than PAU 881.

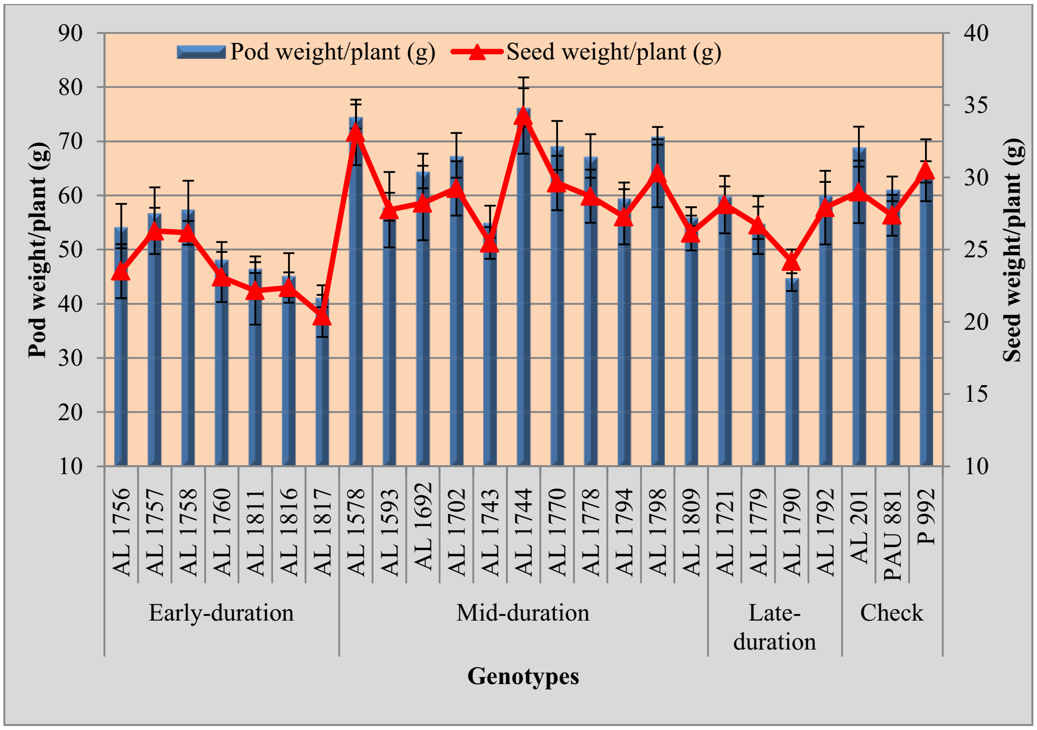


Fig. 8: Pod weight/plant and seed weight/plant in pigeonpea genotypes

ii) Pod set percentage

Maximum pod set percentage was recorded in mid duration genotypes (18.92%) followed by local checks (18.83%) and minimum in early duration group (14.84%) (Table 7). Among early duration group, AL 1756 had maximum (17.25%) pod set percentage and minimum was found in AL 1816 (12.37 %). In mid duration group AL 1770 attained maximum (22.35%) pod set percentage followed by AL 1744 (22.27%) and minimum in AL 1794 (14.49%). Among late duration and check genotypes, AL 1721 and AL 201 had maximum (20.01 and 20.11% respectively) pod set percentage.

Islam *et al* (2010) reported that in legume crops, many flowers are produced but only a few set pods are formed and result in low yield. The main reason for such low yields is abscission of flowers.

Table 7: Pod set percentage and 100-seed weight in pigeonpea genotypes

Genotypes	pod set %	100-seed weight
Early-duration		
AL 1756	17.25±0.95	6.22±0.42
AL 1757	16.31±1.01	6.87±0.16
AL 1758	15.32±1.02	8.31±0.14
AL 1760	16.39±1.19	6.24±0.25
AL 1811	12.69±1.39	6.60±0.34
AL 1816	12.37±0.63	7.23±0.42
AL 1817	13.52±0.42	7.03±0.12
Mean	**14.84±0.52**	**6.93±0.08**
Mid-duration		
AL 1578	21.30±1.97	7.27±0.11
AL 1593	19.99±1.67	6.46±0.10
AL 1692	18.74±0.89	7.48±0.11
AL 1702	17.88±0.99	6.27±0.12
AL 1743	18.20±0.84	6.82±0.18
AL 1744	22.27±0.92	7.20±0.31
AL 1770	22.35±1.30	7.47±0.35
AL 1778	17.14±1.18	7.04±0.06
AL 1794	14.49±0.87	7.15±0.15
AL 1798	17.12±0.98	7.05±0.18
AL 1809	18.61±0.39	6.47±0.34
Mean	**18.92±0.73**	**6.97±0.11**
Late-duration		
AL 1721	20.01±1.68	7.04±0.05
AL 1779	15.42±1.42	6.64±0.20
AL 1790	15.56±0.80	6.56±0.06
AL 1792	15.61±1.39	6.94±0.04
Mean	**16.65±0.29**	**6.80±0.06**
Check		
AL 201	20.11±1.09	7.05±0.10
PAU 881	17.95±0.74	6.62±0.28
P 992	18.43±0.87	7.22±0.22
Mean	**18.83±0.53**	**6.96±0.12**
CD (5%)	**2.35**	**0.33**

iii) Seed weight/plant

Mid duration group produced maximum seed weight/plant than all other groups. Among early duration group, maximum seed weight was recorded in AL 1757 (26.29 g) followed by AL 1758 (26.17g) and minimum was recorded in AL 1817 (20.41) (Fig. 8).

Among mid duration group AL 1744 had maximum (34.29g) seed weight/plant and minimum was recorded in AL 1743 (25.46 g). In late duration group, AL 1790 had minimum (24.18 g) seed weight/plant and was 14% lower than AL 1721, which produced 28.12g seed weight/plant. Among check genotypes P 992 produced maximum (30.50g) seed weight/plant which differed non-significantly from AL 201 (29 g).

iv) 100-seed weight

100-seeds were counted at harvest and expressed in grams. In early duration group, 100-seed weight ranged from 6.22g to 8.31g (Table 7). Maximum 100-seed weight was recorded in AL 1758 (8.31g) while lowest in AL 1756 (6.22g). In mid duration group AL 1770 had maximum (7.47g) 100-seed weight followed by AL 1578 (7.27 g). In long duration group and check genotypes, AL 1721 and P 992 recorded maximum (7.04 and 7.22 g respectively) 100-seed weight.

v) Seed yield (kg/ha)

Yield is an important trait for any crop. It is product of assimilate production and partitioning towards sink (seed) at reproductive phase of crop life cycle. Seed yield was maximum in mid duration group which showed 14% increase over local checks (Fig. 9). Among early group, AL 1757 showed maximum seed yield (1298.33 kg/ha). In mid duration group, maximum seed yield was noticed in AL 1744 (1464.99 kg/ha) followed by AL 1578 (1437.49) and minimum was noticed in AL 1809 (1013.33 kg/ha). Among late and check genotypes AL 1721 and P 992 showed maximum (1237.49 and 1269.46 kg/ha respectively) seed yield.

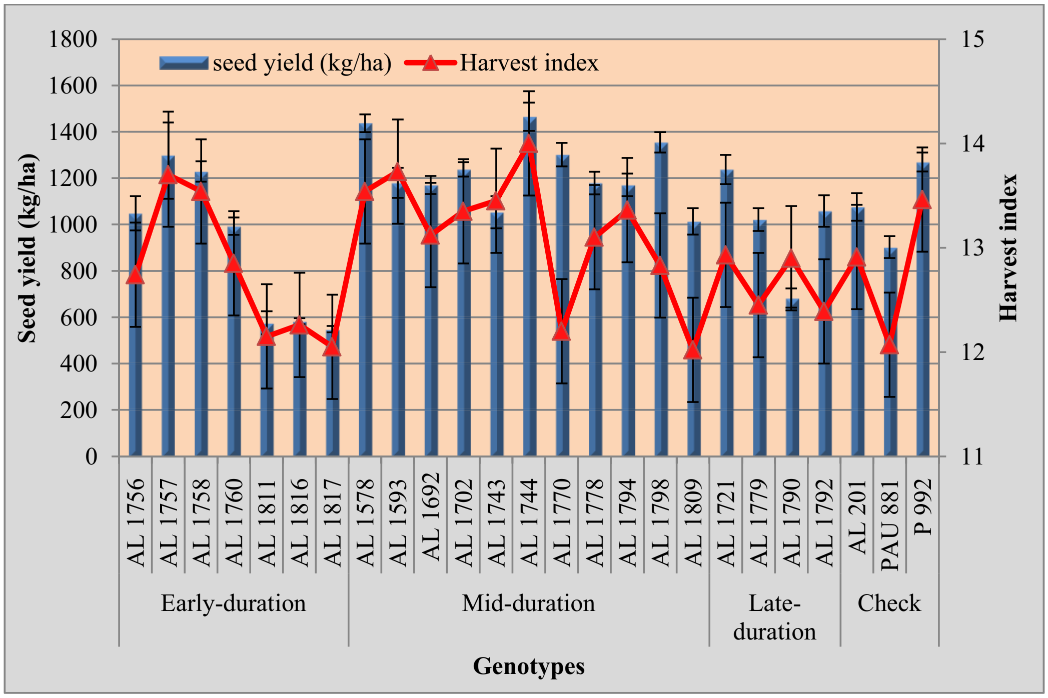


Fig. 9: Seed yield (kg/ha) and harvest index (HI) in pigeonpea genotypes

vi) Harvest index

HI was maximum in mid duration genotypes. In early duration group, genotype AL 1757 had maximum HI (13.70) and minimum was noticed in AL 1817 (12.05) (Fig 9). In mid duration group, maximum HI was noticed in AL 1744 (14.0) followed by AL 1593 (13.73) and AL 1578 (13.54) and minimum was observed in AL 1809 (12.02). Among late and local checks, maximum HI was noticed in AL 1721 (12.93) and P 992 (13.46) respectively.

Seed yield is determined mainly by number of flowers per plant, the percent podset, the number of seeds per pod and seed size in mungbean [*Vigna radiata* (L.) Wilczek] (Mondal 2011b). Most of the yield components show direct influence on grain yield. In legume crops, many flowers are produced, but only a few set pods. Singh *et al* (2008) had also reported genotypic difference with respect to the pods/plant, 100-seed weight and yield.

4.1.5 Correlation and path analysis

Correlation and path analysis is presented in Tables 8 and 9 and Fig. 10. Seed yield is the result of the expression and association of several plant growth components, which contribute additively or help in some conditions in modifying the expression of other traits directly or indirectly. Suffice it to say that the main objective for a plant breeder is to evolve high yielding varieties. It is therefore desirable for plant breeder to know the extent of relationship between yield and its various components, which will inevitably facilitate selection of desirable characteristics. Correlation and path coefficient become necessary tools at the disposal of the breeder. Correlation measures the mutual association between two variables, which aids in determining the most effective procedures for selection of superior genotypes. When there is positive association of major yield traits component, breeding would be very effective but on the reverse, it becomes difficult to exercise simultaneous selection for them in developing a variety. According to Cramer and Wehner (2000b) the goal of indirect selection is to find yield components that have a strong direct effect on fruit number per plant with little negative influence from other yield components. Interestingly, path coefficient analysis can be employed to partition the correlation between yield components and yield into direct and indirect effects. Salahuddin *et al* (2010) affirms that path coefficient analysis provides an effective means of partitioning correlation coefficients into unidirectional pathways and alternative pathways thus permitting a critical examination of specific factors that produce a given correlation, which can be successfully employed in formulating an effective selection programme.

Table 8 revealed that there was significant positive correlation between plant height (0.6534**), CGR (0.7464**), leaf area (0.8273**), SLW (0.7574**), number of pods/plant (0.6031**), pod set % (0.7335**), seed weight/plant (0.839**), 100-seed weight (0.3052*) and harvest index (0.5142*). Further, leaf area which showed higher significant correlation with seed yield also showed positive significant association with SLW (0.539*), pod set

Table 8: Correlation matrix among different characters of pigeonpea

S. No.	Characters	Plant height	CGR (50-100 DAS)	LA	SLW	No. of flowers	No. of pods/plant	Pod set %	Seed wt./plant	100-seed weight	HI
1	Plant height	1									
2	CGR (50-100 DAS)	0.5608**									
3	Leaf area at flowering	0.5415**	0.7968*								
4	SLW at flowering	0.3939*	0.4815*	0.5390*							
5	No. of flowers	-0.1831	-0.9410*	-0.1180	-0.4844*						
6	No. of pods	0.5511**	0.6461**	0.4890*	0.3021*	0.1527					
7	Pod set %	0.6924**	0.6790**	0.5888**	0.5221**	-0.1117	0.9149**				
8	seed wt/plant	0.7626**	0.7602**	0.6877**	0.6889**	-0.2524	0.6985**	0.7898**			
9	100-seed weight	0.2088	0.3086*	0.3240*	0.4518*	-0.2984*	0.1068	0.1254	0.3253*		
10	HI	0.3765*	0.4723*	0.3309*	0.4022*	-0.0902	0.2933*	0.3042*	0.5179**	0.3337*	
11	Yield	0.6536**	0.7464**	0.8273**	0.7574**	-0.2038	0.6031**	0.7336**	0.8349**	0.3053*	0.5143**

** Significant at 1%, * Significant at 5%, CGR=Crop growth rate, LA= Leaf area, SLW= Specific leaf weight, HI= Harvest index

Table 9: Path coefficient analysis showing direct (diagonal) and indirect (non-diagonal) effect of different characters on yield

S.No.	Characters	Plant height	CGR (50-100)	LA	SLW	No. of flowers	No. of Pods	Pod set %	Seed wt./plant	100-seed wt.	HI	Correlation
1	Plant height	**0.1862**	-0.0311	0.2633	0.1885	-0.0013	0.1727	-0.1237	-0.0268	-0.0283	0.0541	**0.6536***
2	CGR (50-100 DAS)	0.1044	**-0.0555**	0.3874	0.2304	-0.0007	0.2025	-0.1213	-0.0267	-0.0419	0.0678	**0.7464***
3	Leaf area at flowering	0.1008	-0.0442	**0.4862**	0.2579	-0.0008	0.1532	-0.1052	-0.0241	-0.044	0.0475	**0.8273***
4	SLW at flowering	0.0733	-0.0267	0.262	**0.4786**	-0.0034	0.0947	-0.0933	-0.0242	-0.0613	0.0577	**0.7574***
5	No. of flowers	-0.0341	0.0052	-0.0544	-0.2318	**0.007**	0.0478	0.02	0.0089	0.0405	-0.0129	**-0.2038**
6	No. of pods	0.1026	-0.0359	0.2377	0.1446	0.0011	**0.3134**	-0.1635	-0.0245	-0.0145	0.0421	**0.6031***
7	Pod set %	0.1289	-0.0377	0.2863	0.2499	-0.0008	0.2867*	**-0.1787**	-0.0277	-0.017	0.0437	**0.7336***
8	seed wt/plant	0.142	-0.0422	0.3344	0.3297	-0.0018	0.2189	-0.1412	**-0.0351**	-0.0442	0.0744	**0.8349***
9	100-seed weight	0.0389	-0.0171	0.1575	0.2162	-0.0021	0.0335	-0.0224	-0.0114	**-0.1357**	0.0479	**0.3053***
10	HI	0.0701	-0.0262	0.1609	0.1925	-0.0006	0.0919	-0.0544	-0.0182	-0.0453	**0.1436**	**0.5143***

Diagonals = Direct effect (bold)
Off diagonals = Indirect effects
(Residual effect = 0.095)

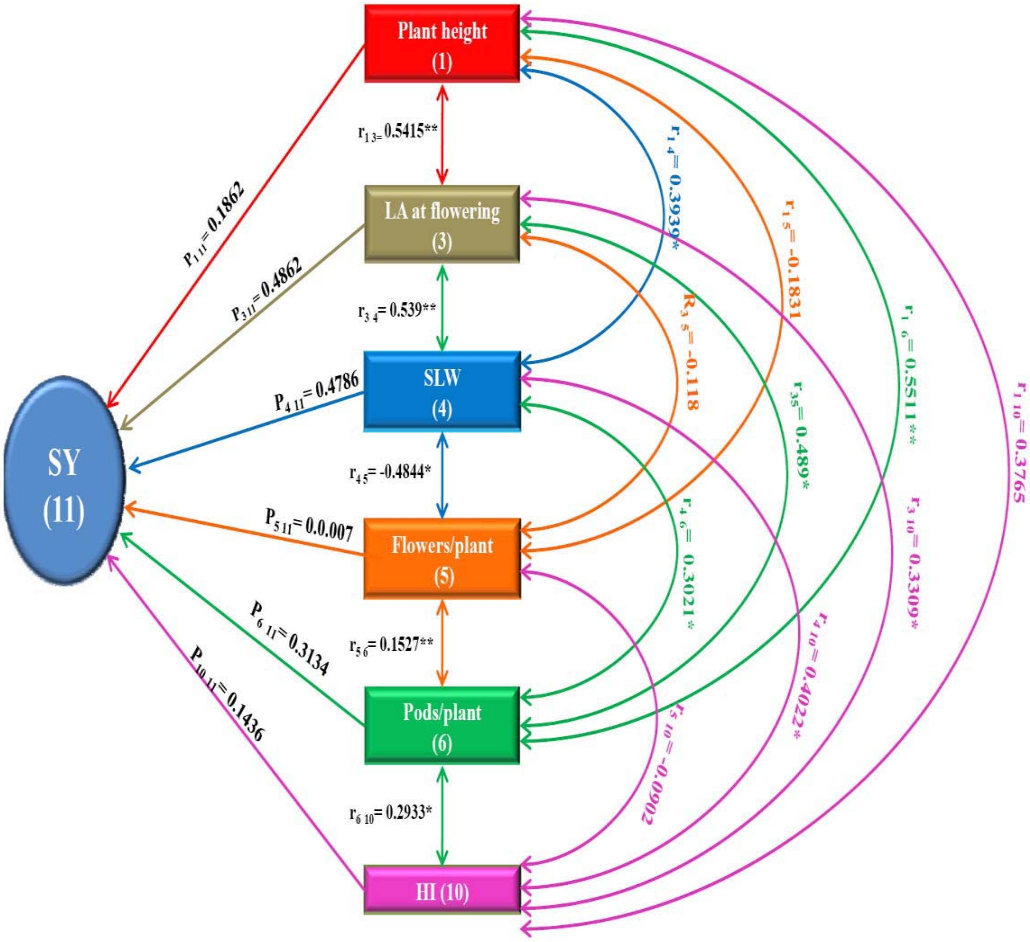


Fig. 10: Path diagram showing the relationship between grain yield and different traits. (Where, single headed arrows indicate direct effects, double headed arrows indicate simple correlation;Significant at 1%)**

percentage (0.5888**) and seed weight/plant (0.6877**). The path analysis (Table 9) showed that different yield traits had varied magnitude of direct effect on yield. It was observed that leaf area had highest direct effect (0.4862**) on yield which was positive. This was followed by SLW (0.4786), number of pods/plant (0.3134) while plant height/plant had negative direct effect. Number of flowers/plant had lowest direct effect (0.007). 100-seed weight contribute indirectly via leaf area and SLW.

Our correlation results showed significantly positive relationships between plant height/plant CGR, leaf area, number of pods/plant, pod set percentage, seed weight/plant, 100-seed weight and HI, respectively. The deduction here is that as the crop increase in height, the number of leaves increase, which could due to the production of more branches. Since the leaves are better exposed to solar radiation owing to the broader leaf area, photosynthesis processes thus increase on the leaf surface with the resultant increase in productivity. This corroborates with the report of Akinyele and Osekita (2006) that height at flowering and final height are vegetative traits that are important for yield determination. Their facilitatory role contributes significantly to final yield and should be considered during selection to improve yield in breeding programme. According to Udensi *et al* (2010) these traits seem to be functioning in tandem with one another. It however implies that direct selection of plant height might be advisable since it result in myriad of effects, including seed yield than direct selection for seed yield itself, particularly if the height has higher heritability than seed yield. The direct effects of leaf area plant^{-1} and SLW, no. of pods/plant, plant height and number of flowers plant^{-1} were positive while 100-seed weight were negative. 100-seed weight contributes indirectly via other traits. These results are in conformity with the reports of Arshad *et al* (2006), Shrivastava *et al* (2001), Khan *et al* (2000) and Singh and Yadava (2000) in soybean. According to Singh and Chaudhary (1985), if the correlation coefficient is positive but the direct effect is negative or negligible, the indirect effects might be the causal factor of correlation. Going by this premise, since correlation for plant height plant^{-1} is 0.6534 while the direct effect is 0.1862, it does mean that alternative pathways (indirect routes) such as leaf area (0.2633), SLW (0.1885) and number of pods/plant (0.1727) should be considered simultaneously for selection.This is in line with the submission of Salahuddin *et al* (2010). The implication is that direct selection programme of these traits would be beneficial for the breeding of this crop. One would have expected the number of pods plant^{-1} to contribute directly to yield. However, our result indicates that alternative pathways via leaf area and SLW should rather be considered for selection indirectly instead of direct selection through number of pods plant^{-1}. The path coefficient result confirms a natural condition one would have imagined. If leaf area plant^{-1} increase then more surfaces will be exposed to radiant solar radiation thus increasing photosynthetic surface with more food production. This led to longer pod, resulting to enhanced seed yield. In order for the crop to produce many seed, plants must accumulate a large photosynthetic area to support the growth of a large number of seeds (Cramer and Wehner, 2000a). From the correlation analysis there was significant positive relationship between the plant height plant^{-1}, leaf area, SLW, number of pods/plant, pod set %, seed weight/plant, 100-seed weigh and HI. This result is in agreement with the result of the direct, indirect and correlation coefficient.

On the basis of experiment I, four mid-duration genotypes (AL 1578, AL 1593, AL 1702 and AL 201) having moderate pod set% and yield were selected for yield improvement by PGRs application.

Experiment II Manipulation of reproductive efficiency of pigeonpea (*Cajanus cajan* L.) genotypes by the use of growth regulators.

4.2.1 Morpho-physiological parameters

4.2.1.1 Plant height

Plant height is an important morphological character in pigeonpea. Basically, plant height is a genetically controlled character, but, several studies have indicated that the plant height is either increased or decreased by the application of synthetic plant growth regulators. However, in the present investigation, significant differences in plant height were noticed.

The data on plant height recorded at different growth stages as influenced by foliar spray of plant growth regulators is presented in Fig. 11. In general plant height increased with the age of the crop till harvest. At vegetative stage genotype AL 1578 showed maximum plant height followed by genotype AL 201.

At flowering stage, there was significant difference among genotypes as well as treatments. The maximum plant height was recorded in control as compared to the foliar application of growth regulators in all the genotypes. On average, genotype AL 1702 showed maximum plant height. Treatment E_1V showed lowest plant height as compared to all other treatments, while C_1V treatment showed maximum plant height (218.5 cm) after control (224.75cm).

At post-flowering stage, the plant height was maximum in control (252 cm) followed by C_1V treatment on mean basis. Both C_1F and C_2F treatments showed significant differences in plant height. Among genotypes AL 1578 showed maximum plant height followed by AL 1702 genotype.

Our results are in close agreement with Campos *et al* (2010), who reported that plant treatment with lower concentration of ethephon resulted in reduced plant height. Ethylene is an inhibitor of cell division, cell expansion and transport of auxin which presents expressive effects on the reduction of stem growth in length; however, it provides its radial expansion and horizontal orientation (Coll *et al* 2001). Sometimes, reduction in plant height may prove beneficial for the crop, especially if it is beneficial for the harvest index (HI), and total

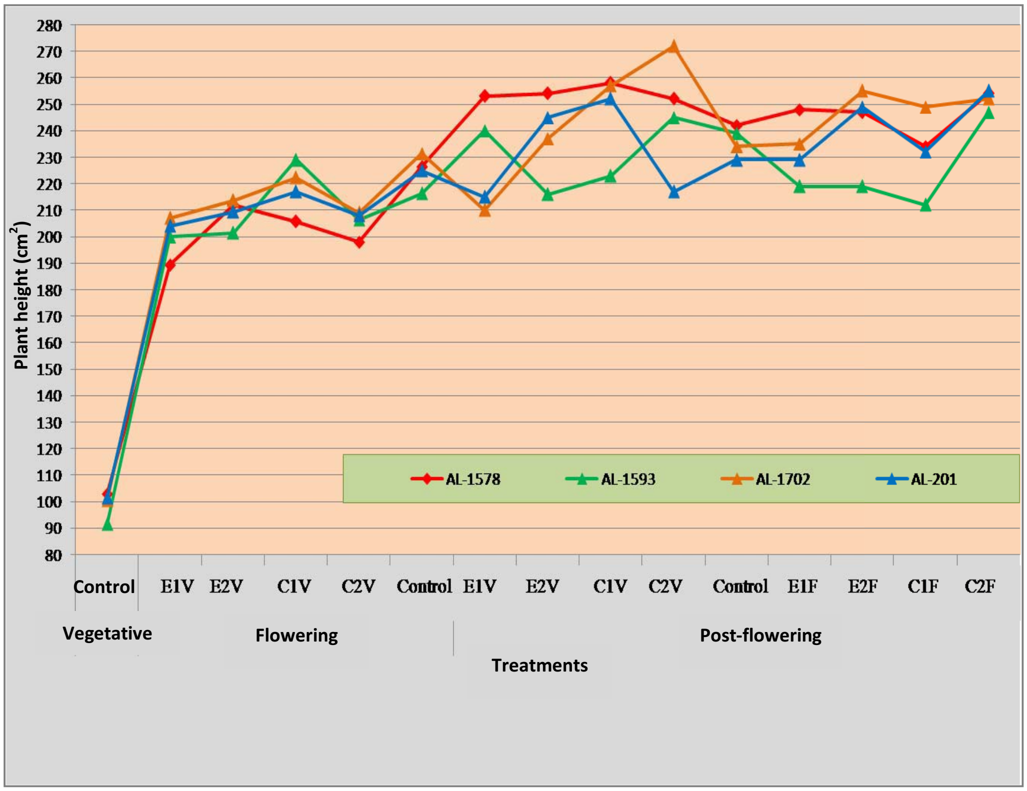


Fig. 11: Effect of foliar application of ethrel and cobalt chloride on plant height in pigeonpea genotypes

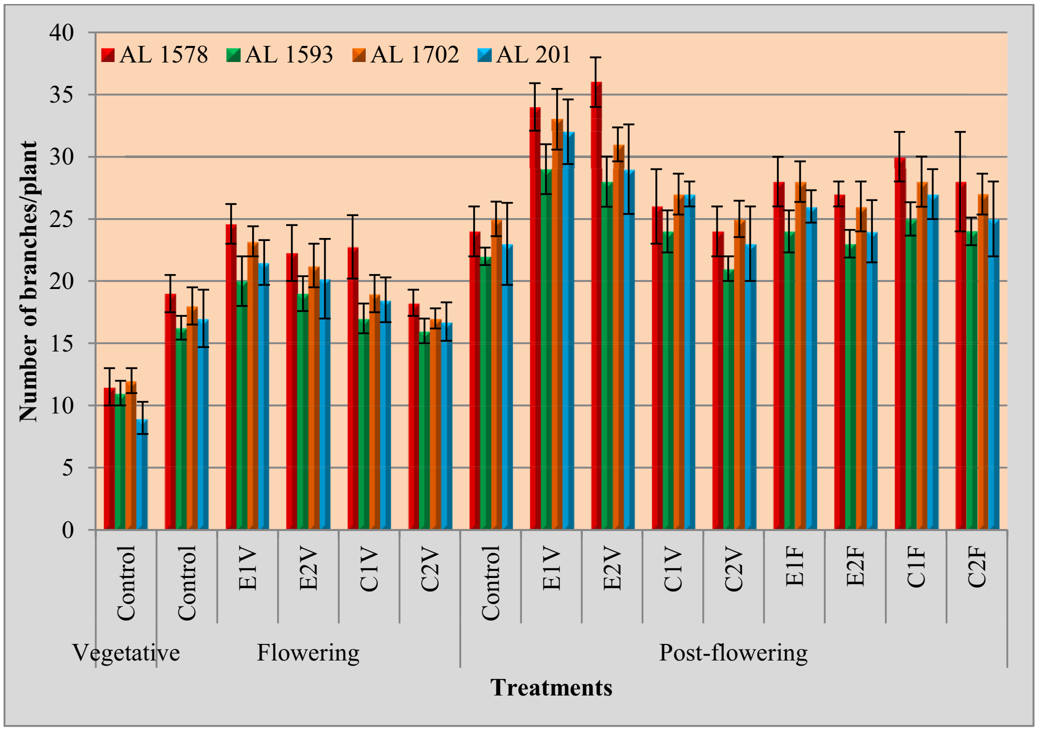


Fig. 12: Effect of foliar application of ethrel and cobalt chloride on number of branches/plant

biomass yield is not reduced and more of photosynthates are partitioned towards the economic sinks. Joshi *et al* (2011) reviewed that ethylene inhibited stem elongation in whole green plants either by inhibiting basipetal IAA translocation or by affecting IAA metabolism in some manner or some other auxin independent action. Also, cobalt above 8 ppm concentration reduced plant height in groundnut (Gad 2012).

4.2.1.2 Number of branches

Number of branches are indicators of pod bearing capacity of a plant. Pod load establishment is dependent on number of primary branches. Improvement in this characteristic is indicative of pod load establishment. Different treatments influenced the branching pattern by enhancing the number of branches. Unlike controls, ethephon treated plants showed profuse branching. The branches were longer and stouter. The number of branches increased linearly with advancement of growth and the stimulatory effect of growth regulators was apparent at all the stages of development.

At vegetative stage, non-significant differences occurred between genotypes for number of branches/plant and genotype AL 1702 had maximum (12) number of branches/plant (Fig. 12) at this stage. However, at flowering stage, there was significant difference among genotypes as well as treatments. On average, E_1V treatment showed significant increase (27.16 %) in number of branches/plant over control followed by E_2V (17.72 %). Control and C_2V treatment differed non-significantly from each other at this stage. At post-flowering stage, E_1V treatment showed 36.17% increase in number of branches followed by E_2V (31.91 %) and C_1F (17.0%) treatment as compared to control (on mean basis). Treatments C_1V, C_1F and C_2F differed non-significantly from each other. Both at flowering and post-flowering stage, AL 1578 showed maximum number of branches/plant. Genotype AL 1578 showed maximum number of branches/plant in E_2V treatment (36) as compared to E_1V treatment (34).

The branches per plant differed significantly among the treatments and increased due to application of PGRs. This could be the result of increased metabolic and divisional activities in shoot apical meristems. Several physical, genetic and chemical factors are known to exert their influence on meristematic activity in shoot apical meristem. A specific balance of gibberellins, auxins and cytokinins is known to exert their influence on meristematic activity in shoot apical meristem (Taiz and Zeiger 2006). Maximum increase in number of branches by ethrel treatment might be due to inhibition of apical bud dominance and breaking of lateral bud dormancy. Our results are in agreement with Campos *et al* (2010). An increase in ethylene level was observed after removing apical bud in soybean plants, which provided higher cytokinin content to the development of lateral buds and hence increase in number of branches (Tancredi 2004).

4.2.1.3 Growth efficiency

i) Relative growth rate (RGR)

Relative growth rate was calculated between 50-100 DAS (stage I) and 100-145 DAS (stage II). Values of RGR were initially high at stage I then decreased at stage II as compared to stage I (Fig. 13).

At stage I, lower concentration of ethrel (E_1V) showed maximum RGR (0.082 g plant^{-1}day^{-1}) followed by E_2V treatment (0.080 g plant^{-1}day^{-1}), which was 17.14% and 12.5% higher than control. Among the genotypes, AL 1578 had maximum RGR (0.079 g plant^{-1} day^{-1}) followed by AL 201 (0.077) at this stage. At stage II, maximum RGR was found in C_1F treatment (0.0233 g plant^{-1}day^{-1}) followed by E_1F (0.0215 g plant^{-1}day^{-1}) and E_1V (0.0205 g plant^{-1}day^{-1}). Lowest value of RGR was found in control plants. Genotype AL 1578 had highest RGR (0.0213 g plant^{-1}day^{-1}) followed by AL 1702 (0.0209 g plant^{-1}day^{-1}) and lowest was found in AL 1593 (0.0187 g plant^{-1}day^{-1}).

ii) Crop growth rate (CGR)

The crop growth rate showed an increasing trend as the crop advanced towards maturity. Saishankar (2001) also reported that CGR in greengram was low at vegetative stage but increased with the advancement of growth.

CGR varied significantly among treatments and genotypes (Fig. 14). At stage I, E_1V treatment showed significant increase (17.47%) over control plants. Lowest CGR was shown by control plants followed by C_2V treated plants. On average of all treatments, genotype AL 1578 showed 5.46% increase in CGR as compared to AL 201. At stage II, CGR was more as compared to stage I. Maximum CGR was found in C_1F treated plants (19.024 g m^{-2} day^{-1}) followed by E_1V (18.54 g m^{-2} day^{-1}), E_2V (17.425 g m^{-2} day^{-1}) and E_2F (17.420 g m^{-2} day^{-1}) treatments. Lowest CGR was shown by control plants (14.515 g m^{-2} day^{-1}). Similar to stage I, AL 1578 genotype showed maximum CGR during stage II also.

RGR represents the increase in dry matter per unit of dry matter already present per unit time. The decline in RGR with the advancement in crop growth could be due the decline in the rate of dry matter production. The increase in RGR due to growth regulators would be due to the effectiveness of these chemicals in increasing not only the total dry matter but also the rate of increment in total dry matter. This could also be attributed to increased photosynthetic efficiency by increasing leaf thickness and retaining more chlorophyll content and efficient translocation (Jirali 2001). CGR was associated with the amount of extent of light energy intercepted and the photosynthetic efficiency of the leaf. The increase in RGR, CGR at a particular stage depicts the plant responses to the PGRs at that particular stage and the maximum efficiency of the crop growth can be attained. Karim and Fattah (2007) reported that peak values of CGR were found during pod development stage and foliar application of growth regulators was most effective in increasing CGR as compared to control. Rahman *et al* (2004) concored that increase in CGR due to the application of growth regulators was certainly the result of increase in dry matter production with time course. Increase in RGR

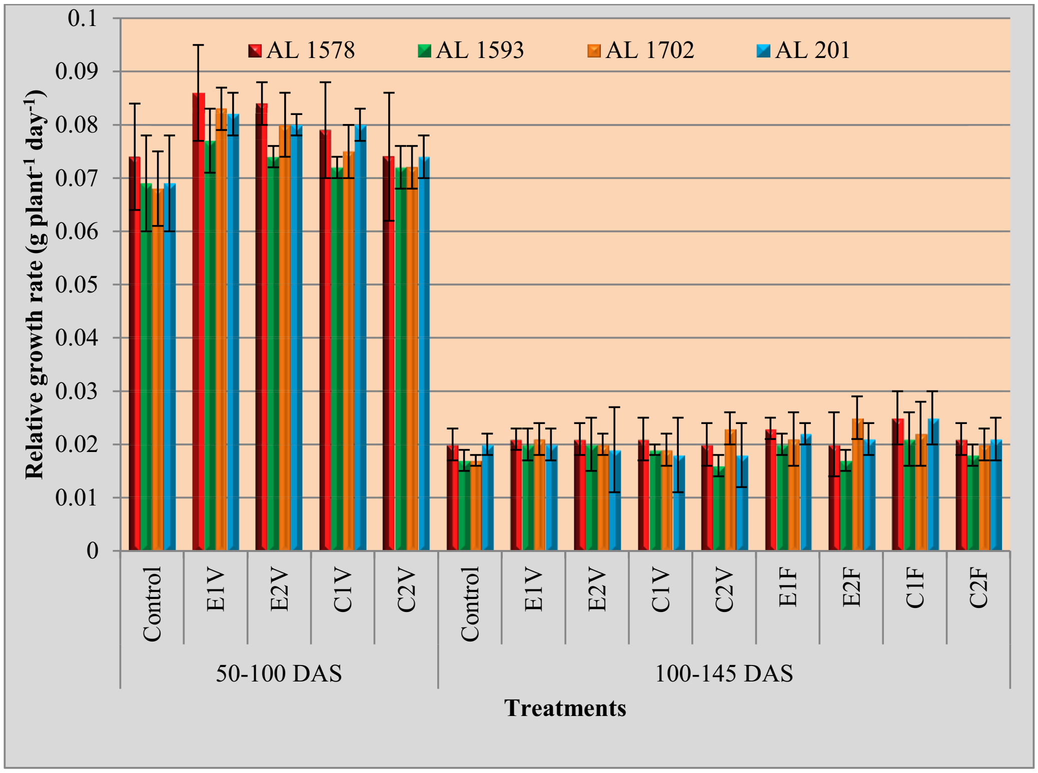


Fig. 13: Effect of foliar application of ethrel and cobalt chloride on relative growth rate (RGR)

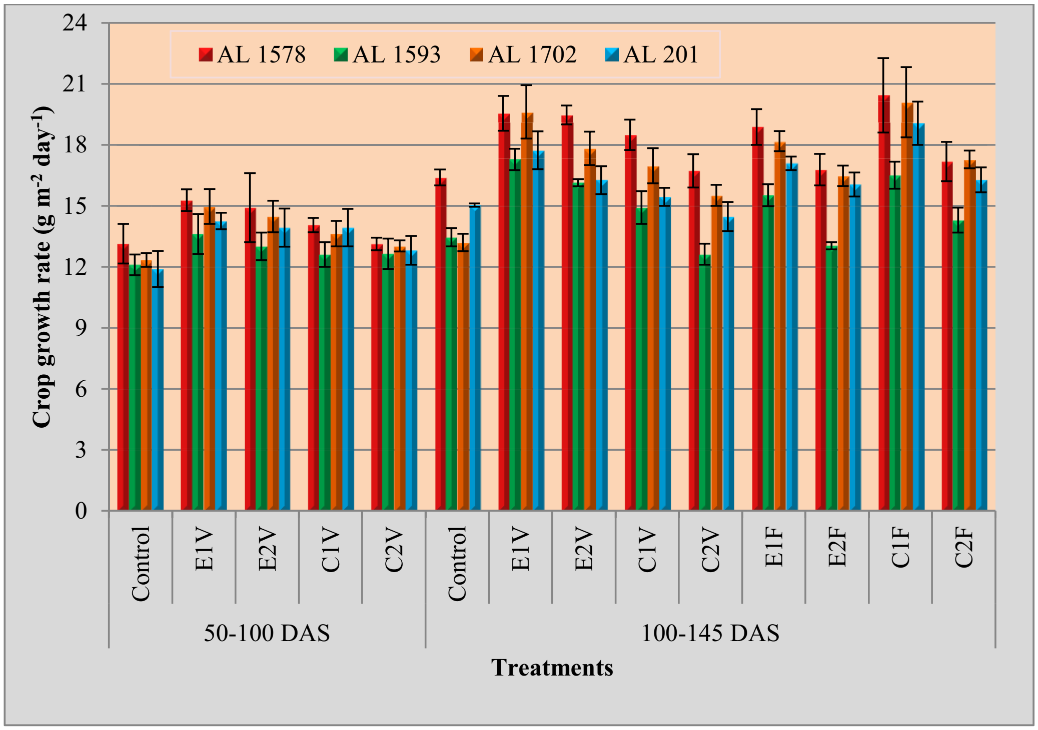


Fig. 14: Effect of plant growth regulators on crop growth rate in pigeonpea genotypes

and CGR by growth retardants and promoters sprayed at particular stage was also observed by Prabhu (2000).

4.2.1.4 Leaf characteristics

i) Leaf area

Leaf area is determined by phenology, stem morphology, rates of leaf emergence and potential leaf size. With advancing growth stage, the leaf abscission results in lower number of leaves, reduced leaf area of the plant and ultimately lesser photosynthetic area. Leaf area per plant indicated significant differences among the treatments at all the stages. Leaf area was more due to treatments as compared to control (Fig 15).

At vegetative stage, maximum leaf area was observed in AL 1578 (2530.85 cm^2) while minimum leaf area was observed in AL 1593 (2265.42 cm^2). At flowering stage, E_1V treatment caused maximum increase in leaf area which was 12.72% more as compared to control on average of all genotypes (Fig. 15). Genotype AL 1578 produced maximum leaf area/plant in all the treatments. At post flowering stage, E_2V treated plants (mean of all genotypes) had maximum (2849.55 cm^2) leaf area/plant followed by E_1V (2843.72 cm^2), which was 17.90 and 17.66 % higher, respectively, than that of control plants. Like flowering stage, AL 1578 produced highest leaf area at this stage also which was 14% higher than AL 201 genotype.

Ethrel when sprayed at pre-flowering stages has a positive effect on cell division leading to enhanced leaf expansion. Saxena *et al* (2007) also found a significant increase in leaf area at all stages with the application of lower concentration of ethrel. Leaf area increased with increase in time to a maximum coinciding with maximum top growth and steady decline at later stages (Jirali 2001). Bhat *et al* (2010) reported that lower concentration of ethrel at specific stage promoted expansion of primary leaves, while higher concentration of ethrel showed a reduction in the area of mustard leaves. The increase in leaf area by cobalt application could be due to increase in number and size of cells. Gad (2005) pointed out that low level of applied cobalt increased the leaf area as well as the size of the chloroplast in tomato plants. Similar results were reported by Vijayarengan (2012) in cowpea following cobalt application.

ii) Leaf area index (LAI)

Photosynthesis depends upon the leaf area index besides the canopy structure and photosynthetic rate. The variation in LAI was studied at the different stages of sampling. Maximum value for LAI was observed at flowering stage (Fig. 16).

At vegetative stage, AL 1578 recorded maximum (1.80) LAI followed by AL 201 (1.67) and lowest value of LAI was found in AL 1593 (1.43). At flowering stage there was significant difference among treatments. The highest LAI was found in E_1V (4.57) treated plants (mean of all genotypes) followed by E_2V (4.31) and lowest value of LAI was attained in control plants. At post-flowering stage, all genotypes showed decrease in LAI under all

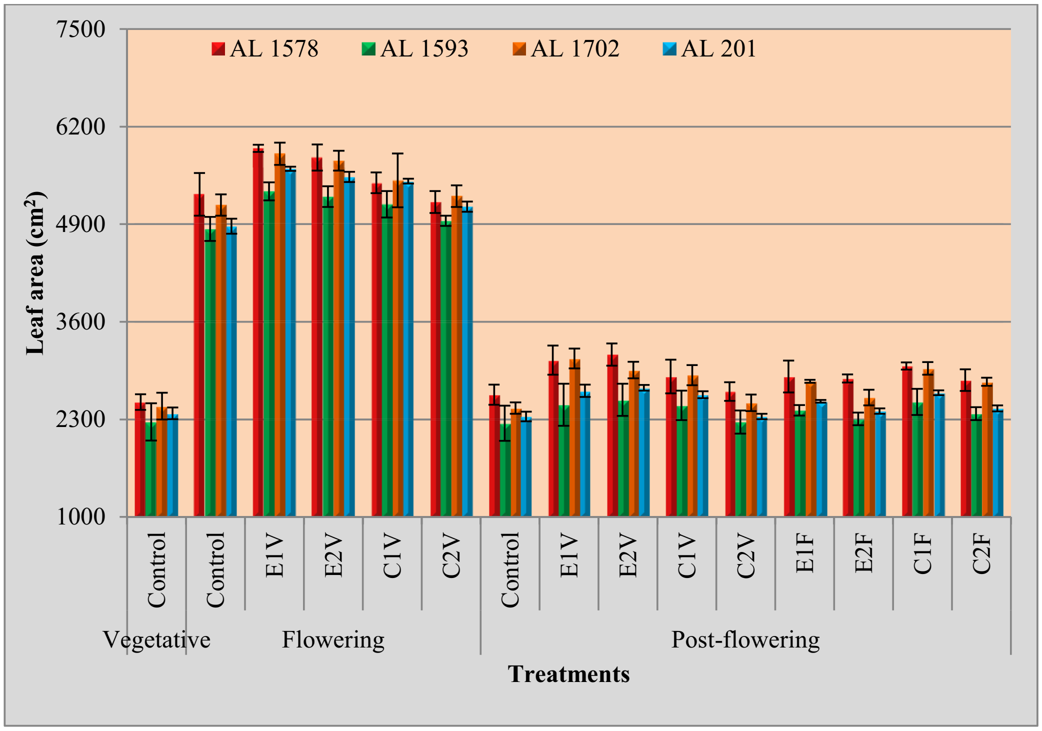


Fig. 15: Effect of foliar application of ethrel and cobalt chloride on leaf area in pigeonpea genotypes

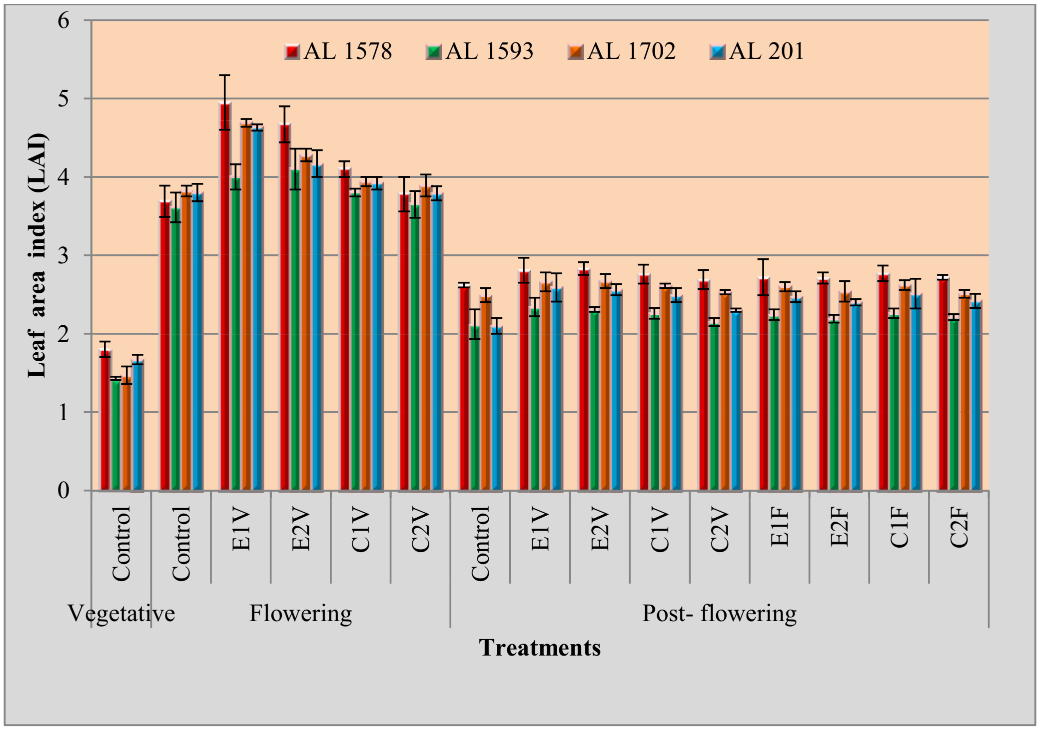


Fig. 16: Effect of foliar application of ethrel and cobalt chloride on leaf area index in pigeonpea genotypes

treatments including control due to senescence of matured leaves. Maximum LAI was observed in E_1V treatment (2.60) and lowest in control plants. At all stages, AL 1578 registered maximum LAI.

Our results are in agreement with Mir *et al* (2008), who observed that foliar spray of ethephone with 80 kgN/ha increased LAI, net photosynthesis rate, chlorophyll content and stomatal conductance in brassica. Devi *et al* (2011) also reported increase in leaf area index by PGRs application. Lower concentration of cobalt significantly increased LAI in groundnut (Basu 2011).

iii) Specific leaf weight (SLW)

In the present study, the SLW increased from vegetative stage till harvest (Fig. 17). At vegetative stage, genotype AL 201 recorded maximum (5.08 mg cm^{-2}) SLW than all other genotypes. Minimum SLW was recorded in AL 1593 (4.63 mg cm^{-2}). At flowering and post-flowering stage, E_1V treatment (mean of all genotypes) registered maximum SLW which was 6.739 and 7.241 mg cm^{-2}, respectively. Minimum SLW was recorded in control plants. Among the genotypes AL 1578 recorded highest SLW under all treatments at both these stages.

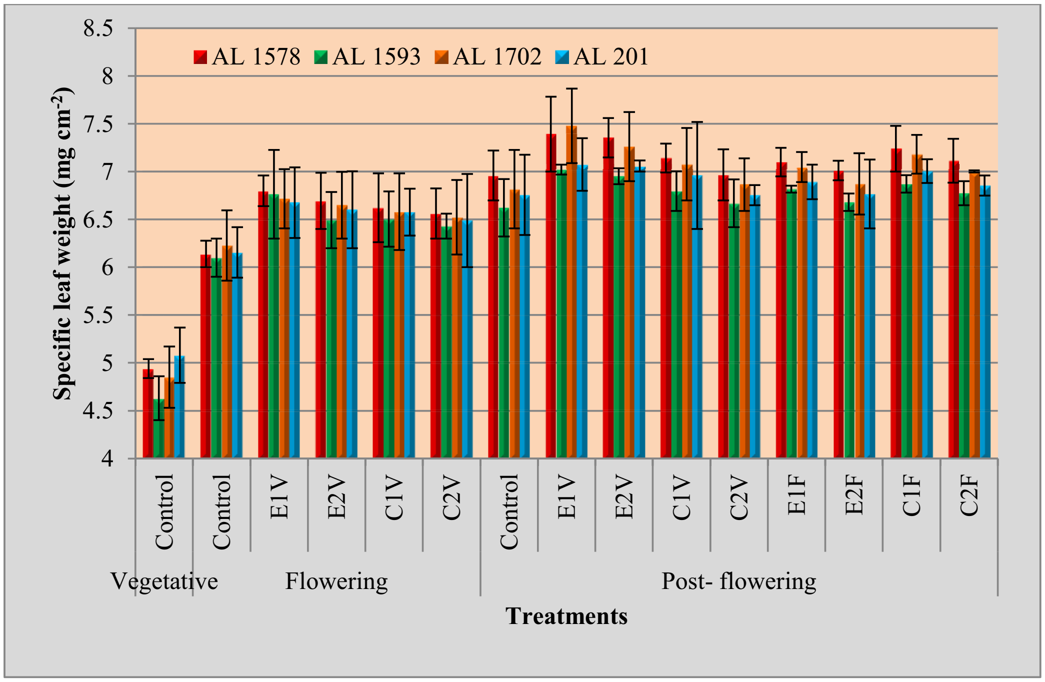


Fig. 17: Effect of foliar application of ethrel and cobalt chloride on specific leaf weight in pigeonpea genotypes

Prakash and Ganeshan (2000) reviewed that even though all the growth regulators and micronutrients increased SLW, it was much more pronounced with growth retardants and growth promoters recorded lower SLW, because they produced thinner leaves. In case of growth retardants, increased SLW may be attributed to the increase in leaf area and hence an increased accumulation of assimilates in the leaf. Thicker leaves allow a greater concentration of photosynthetic apparatus per unit leaf area. While thinner leaves can intercept more light. Thus, variation in leaf thickness can have a major influence on crop growth and productivity. Increase in SLW by application of growth retardents at specific time was also observed by Reddy *et al* (2009).

iv) Specific leaf area (SLA)

SLA is inverse of specific leaf weight. SLA indicated significant differences among all the treatments at all the stages. In general SLA decreased as the age of the crop advanced. SLA was maximum at vegetative stage and decreased thereafter towards harvest (Table 10). At flowering and post-flowering stage, maximum SLA was calculated in control plants followed by C_2V treated plants.

Table 10: Effect of foliar application of ethrel and cobalt chloride on Specific leaf area in pigeonpea genotypes

Treatments	Genotypes				
	AL 1578	AL 1593	AL 1702	AL 201	Mean
			Vegetative stage		
Control	0.202±0.018	0.216±0.016	0.206±0.026	0.197±0.003	**0.205±0.016**
CD (5%)	0.01				
			Flowering stage		
Control	0.163±0.005	0.164±0.009	0.161±0.029	0.162±0.008	**0.163±0.013**
E_1V	0.147±0.003	0.148±0.008	0.149±0.011	0.150±0.040	**0.148±0.016**
E_2V	0.149±0.008	0.154±0.005	0.150±0.020	0.151±0.008	**0.151±0.010**
C_1V	0.151±0.004	0.154±0.006	0.152±0.018	0.152±0.003	**0.152±0.008**
C_2V	0.152±0.002	0.155±0.004	0.153±0.027	0.154±0.006	**0.154±0.010**
Mean	**0.152±0.006**	**0.155±0.008**	**0.153±0.022**	**0.154±0.011**	
CD (5%)	Genotypes (G) = 0.012, Treatments (T) = 0.02, G X T = 0.001				
			Post-flowering stage		
Control	0.144±0.005	0.151±0.007	0.147±0.003	0.148±0.002	**0.147±0.004**
E_1V	0.135±0.005	0.142±0.009	0.134±0.015	0.141±0.008	**0.138±0.009**
E_2V	0.136±0.006	0.144±0.004	0.138±0.008	0.142±0.004	**0.140±0.006**
C_1V	0.140±0.001	0.147±0.009	0.141±0.004	0.144±0.005	**0.143±0.005**
C_2V	0.143±0.003	0.150±0.040	0.146±0.003	0.148±0.005	**0.147±0.013**
E_1F	0.140±0.002	0.147±0.006	0.142±0.006	0.145±0.005	**0.144±0.005**
E_2F	0.143±0.003	0.150±0.04	0.146±0.004	0.148±0.002	**0.146±0.012**
C_1F	0.138±0.006	0.145±0.025	0.139±0.004	0.143±0.004	**0.141±0.010**
C_2F	0.141±0.007	0.148±0.012	0.143±0.006	0.146±0.003	**0.144±0.007**
Mean	**0.140±0.004**	**0.147±0.0181**	**0.142±0.006**	**0.145±0.0045**	
CD (5%)	Genotypes (G) = 0.003, Treatments (T) = 0.006, G X T = 0.03				

4.2.2 Photosynthesis and related parameters

Photosynthesis was measured at vegetative, flowering and pod-filling stage using fully expanded leaf (the third from top) with a portable photosynthsis system (LICOR) assembled with an Infra Red Gas Analyzer (IRGA). Net photosynthetic rate and other related parameters were available as computer output attached to system.

i) Photosynthetic rate

The production of biomass and grain yield of pulse crops can largely be accounted for by photosynthesis during growth and maturation. It is generally considered that for high yield, high photosynthetic potentials are necessary. Net photosynthesis increased from vegetative to flowering stage and then declined towards maturity. Different treatments have preferential effect on rate of photosynthesis.

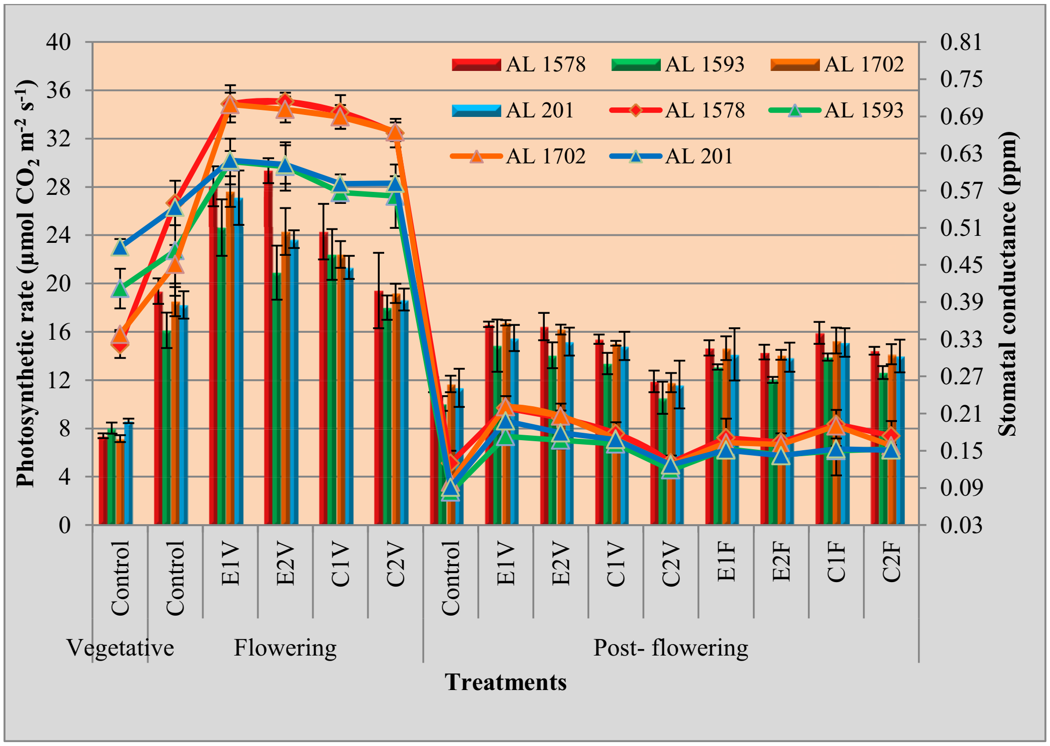


Fig. 18: Effect of foliar application of ethrel and cobalt chloride on photosynthesis and stomatal conductance in pigeonpea genotypes

At vegetative stage, genotype AL 201, recorded highest rate of photosynthesis (8.63 μmol CO_2 m^{-2} s^{-1}) than all other genotypes (Fig. 18). At flowering stage, all treatments recorded higher rate of photosynthesis as compared to control. On average of all genotypes, highest rate of photosynthesis was recorded in E_1V (26.85 μmol CO_2 m^{-2} s^{-1}) treated plants, which showed 48.75% increase over control. Among the genotypes, AL 1578 recorded highest rate of photosynthesis in all treatments applied except in treatment C_2V where AL 1593 showed 10.45% higher net photosynthetic rate as compared to AL 1578.

At pod development stage, net rate of photosynthesis decreased in all genotypes and treatments. Among the treatments, E_1V treatment (on mean basis) recorded maximum rate of photosynthesis (15.93 µmol CO_2 m^{-2} s^{-1}) followed by E_2V (15.47 µmol CO_2 m^{-2} s^{-1}) and C_2F (15.05 µmol CO_2 m^{-2} s^{-1}), which showed 42.49, 38.37 and 34.61 % increase in net photosynthetic rate respectively over control. While minimum was shown by C_2V treatment (11.46 µmol CO_2 m^{-2} s^{-1}). On mean basis, net photosynthetic rate was maximum in AL 1578 (14.58 µmol CO_2 m^{-2} s^{-1}) followed by AL 1702 (14.40 µmol CO_2 m^{-2} s^{-1}) and was recorded minimum in AL 1593 (12.73 µmol CO_2 m^{-2} s^{-1}).

Mir *et al* (2010) reported that the induction of ethylene biosynthesis might be associated with the application of ethrel, which is a known source of ethylene. This effect of ethrel led to the emergence and formation of leaves with enhanced total leaf area of plant. Higher leaf area resulted in more solar radiation being retained and enhanced net photosynthetic rate (Pn) and total dry matter production. The increase in photosynthesis with ethrel has also been reported by Pua and Chi (1993) and Khan *et al* (2000). Exogenous application of ethrel enhanced photosynthesis of *Brassica juncea* under irrigated and non-irrigated conditions (Khan *et al* 2000, Khan 2004). The dry matter produced was efficiently translocated towards the developing pods resulting in increase in the seed yield. Jaleel *et al* (2009) reported that increased chlorophyll content at lower level of cobalt was obviously due to better growth and hence resulted in high photosynthetic activity.

ii) Internal CO_2 concentration (Ci):

Ci values differed in all genotypes and was maximum in genotype AL 1578 while among treatments none of the treatment showed significant effect over control (Table 11). At flowering stage E_1V treatment showed highest Ci (307.02 ppm) followed by control (305.74 ppm) and lowest was recorded in C_2V treatment (285.89 ppm). At post flowering stage Ci was less as compared to flowering stage. Control showed highest Ci (289.95) than all treatments applied (289.95 ppm).

iii) Stomatal conductance (mol m^{-2} s^{-1}):

Various treatments exhibited significant differences in stomatal conductance. It showed a similar trend as that of net photosynthetic rate (Fig. 18). At flowering stage, on mean basis, treatment E_1V showed maximum Cs followed by E_2V and C_1V treatment, which showed 31.74, 30.75 and 25.59 % increase over control. At post flowering stage, treatment E_1V recorded maximum Cs followed by E_2V (0.189 mol m^{-2} s^{-1}), C_1F (0.171 mol m^{-2} s^{-1}) and C_1V (0.169 mol m^{-2} s^{-1}) treatments. Minimum value of Cs was recorded in control (0.101 mol m^{-2} s^{-1}) followed by C_2V (0.126 mol m^{-2} s^{-1}). In all the treatments, genotype AL 1578 showed maximum Cs followed by AL 1702.

Table 11: Effect of ethrel and cobalt chloride on internal CO_2 concentration in pigeonpea genotypes

Treatments	**Genotypes**				
	AL 1578	**AL 1593**	**AL 1702**	**AL 201**	**Mean**
			Vegetative stage		
Control	320.20±5.40	289.60±17.60	311.30±8.70	288.14±7.86	**302.31±9.89**
CD (5%)	31.20				
			Flowering stage		
Control	325.42±3.81	294.30±21.30	310.83±5.17	292.41±7.59	**305.74±9.47**
E_1V	327.14±15.14	283.80±6.80	318.45±6.55	298.68±7.32	**307.02±8.95**
E_2V	328.61±14.01	274.34±7.34	312.74±5.26	289.19±7.81	**301.22±8.61**
C_1V	314.45±13.15	274.13±4.13	312.63±10.37	282.40±9.60	**295.90±9.31**
C_2V	299.10±14.10	269.32±3.68	305.67±11.33	269.49±9.51	**285.89±9.65**
Mean	**318.94±12.04**	**279.18±8.65**	**312.06±7.74**	**286.43±8.37**	
CD (5%)	Genotypes (G) = 2.07, Treatments (T) = 0.11, G X T = 1.24				
			Post-flowering stage		
Control	289.32±17.32	296.48±10.48	292.43±8.57	281.58±5.42	**289.95±10.45**
E_1V	301.80±14.80	274.31±10.31	312.40±12.60	227.91±11.09	**279.10±12.20**
E_2V	310.42±10.42	276.38±7.62	298.6±11.40	229.40±8.60	**278.70±9.51**
C_1V	305.80±18.80	272.92±10.92	289.35±9.65	224.14±6.86	**273.05±11.56**
C_2V	288.34±13.34	274.67±4.33	287.03±9.97	273.46±9.54	**280.87±9.29**
E_1F	301.56±4.56	264.72±4.28	286.12±5.88	220.17±16.83	**268.14±7.89**
E_2F	289.60±16.60	270.43±8.57	273.40±15.60	222.71±8.29	**264.03±12.26**
C_1F	305.14±19.14	277.14±8.14	300.18±9.82	224.8±10.20	**276.81±11.82**
C_2F	283.60±6.60	269.97±5.97	281.23±10.77	221.56±13.44	**264.09±9.19**
Mean	**297.29±13.51**	**275.22±7.85**	**291.19±10.47**	**236.19±10.03**	
CD (5%)	Genotypes (G) = 0.062, Treatments (T) = 0.083, G X T = 0.14				

Our results are in agreement with Hossain *et al* (2009). Kumar *et al* (2005) reported that increase in rate of photosynthesis is due to increased stomatal aperture, which facilitate more CO_2 conductance. Pierik *et al* (2006) reviewed that enhanced stomatal conductance at a range of ethylene concentrations stimulated photosynthesis. Cobalt at lower concentrations increased the stomatal conductance, thereby increasing rate of photosynthesis (Jaleel *et al* 2009).

iv) Chlorophyll kinetics

Chlorophyll fluorescence is widely accepted as an indication of the energetic behavior of a photosynthetic system. Photosynthetic activity was studied in terms of quantum yield (Fv/Fm) and total chlorophyll. The Fv/Fm ratio can be considered as a measure of the quantum efficiency of the electron transport in PSII. By measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be obtained (Table 12).

Table 12: Effect of ethrel and cobalt chloride on Chlorophyll kinetics (Fv/Fm) in pigeonpea genotypes

Treatments	Genotypes				
	AL 1578	**AL 1593**	**AL 1702**	**AL 201**	**Mean**
			Vegetative stage		
Control	0.720	0.726	0.670	0.721	**0.709**
CD (5%)	0.36				
			Flowering stage		
Control	0.764	0.759	0.760	0.764	**0.762**
E_1V	0.799	0.771	0.783	0.782	**0.784**
E_2V	0.795	0.770	0.781	0.781	**0.782**
C_1V	0.790	0.768	0.779	0.776	**0.778**
C_2V	0.783	0.761	0.773	0.770	**0.772**
Mean	**0.786**	**0.766**	**0.775**	**0.775**	
CD (5%)	Genotypes (G) = 0.008, Treatments (T) = 0.011, G X T = 0.018				
			Post-flowering stage		
Control	0.611	0.602	0.606	0.606	**0.606**
E_1V	0.650	0.639	0.640	0.641	**0.643**
E_2V	0.648	0.636	0.638	0.637	**0.640**
C_1V	0.641	0.632	0.634	0.633	**0.635**
C_2V	0.628	0.622	0.624	0.625	**0.625**
E_1F	0.638	0.631	0.632	0.631	**0.633**
E_2F	0.632	0.625	0.628	0.627	**0.628**
C_1F	0.648	0.637	0.636	0.635	**0.639**
C_2F	0.637	0.629	0.631	0.631	**0.632**
Mean	**0.637**	**0.628**	**0.630**	**0.630**	
CD (5%)	Genotypes (G) = 0.009, Treatments (T) = 0.011, G X T = 0.019				

At vegetative stage, AL 1593 genotype had highest photochemical efficiency (0.726) and lowest was recorded in AL 1702 (0.670). While at flowering stage, AL 1578 recorded maximum photochemical activity (0.786) in all treatments including control. Among the treatments E_1V (0.784) followed by E_2V (0.782) recorded maximum photochemical activity. At post-flowering stage, decrease in photochemical efficiency was recorded in all genotypes and treatments. The maximum decrease in photochemical efficiency occurred in control plants.

v) Total chlorophyll content

Pigments such as chlorophylls are needed by plants to absorb sufficient amount of light through photosynthesis. During leaf development, the level of pigments in the leaves increases to provide energy through photosynthesis. Chlorophyll is an important bio-constituent for productivity of the crop plants. Chlorophyll catabolism is the first step of degeneration during senescence but most if not all of enzyme machinery is present in the cell at a basal level before senescence begins. However, chlorophyll catabolism increases from a low baseline on induction of senescence. There was a remarkable decrease in the chlorophyll content at post-flowering stage indicating initiation of senescence process towards maturity (Table 13).

Total chlorophyll content first increased upto flowering stage and then decreased towards maturity. Table 13 revealed that at vegetative stage, genotype AL 1593 showed maximum chlorophyll content (3.26 mg g^{-1} FW) followed by AL 1578 (3.10 mg g^{-1} FW) and minimum chlorophyll content was recorded in AL 1702 (2.94 mg g^{-1} FW). At flowering stage, maximum chlorophyll content was estimated in AL 1578 in all treatments. Among treatments E_1V recorded maximum chlorophyll content followed by E_2V and C_1V. Similar trend was seen at post-flowering stage.

Maximum chlorophyll content was seen at flowering stage and then further decreased as the crop moves towards maturity indicating the ageing induced differential rate of degradation of leaf pigments. Loss of green colour is the visible symptom of senescence, the progress of which is usually measured by determining the amount of chlorophyll. All the treatments showed increased total chlorophyll content than control.

Campos *et al* (2010) proposed that in soybean ethephone delayed the phenological cycle of the crops and maintains chlorophyll content high in the end of the crop cycle, which reinforces the fact that cytokinins inhibit the degradation of chlorophyll on plants. The non-degradation of chlorophyll on the tissue maintains them photosynthetically active, thus, it provided larger quantity of organic matter to the grain filling, leading to the formation of heavier grains, and consequently, increasing grain productivity. Similar results were reported by Devi *et al* (2011). Jaleel *et al* (2009) reported that lower concentration of cobalt increased chlorophyll content in *Zea mays*. Similar effect of cobalt was reported by Jayakumar (2009) in soybean.

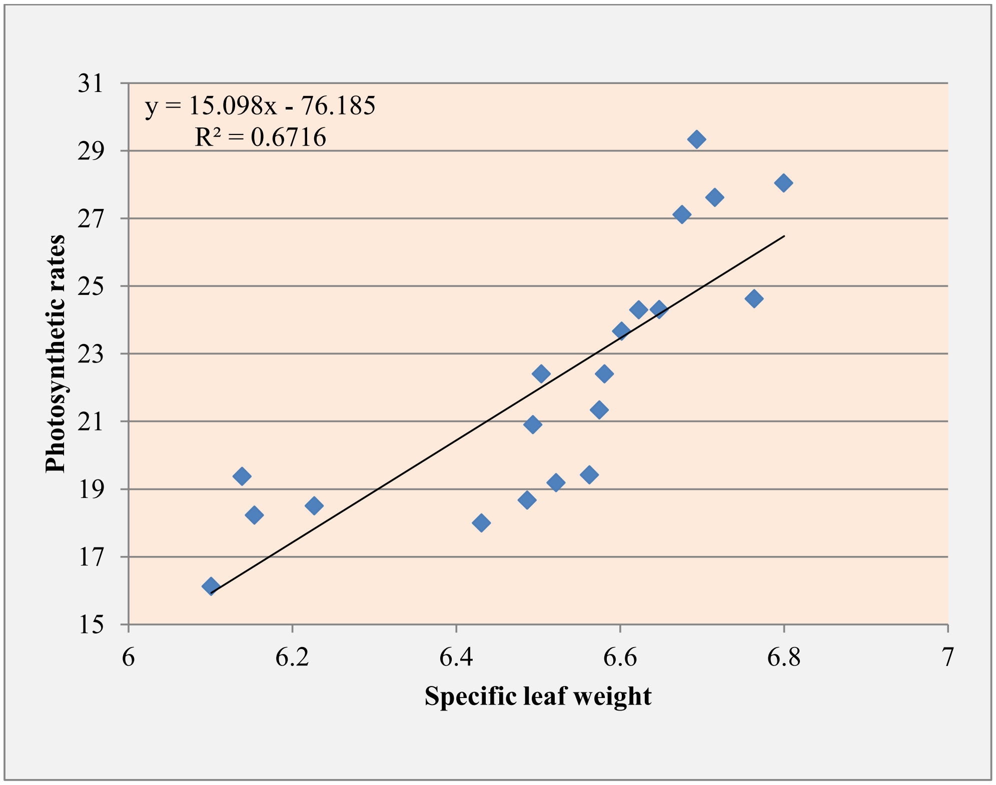
Table 13: Effect of ethrel and cobalt chloride on total chlorophyll content in leaves of pigeonpea genotypes

Treatments	Genotypes				
	AL 1578	**AL 1593**	**AL 1702**	**AL 201**	**Mean**
			Vegetative stage		
Control	3.10	3.26	2.94	3.03	**3.08**
CD (5%)	0.20				
			Flowering stage		
Control	4.49	4.00	4.28	4.20	**4.24**
E_1V	6.00	5.34	5.83	5.60	**5.69**
E_2V	6.10	5.30	5.60	5.52	**5.63**
C_1V	5.63	5.18	5.24	5.19	**5.31**
C_2V	5.16	4.73	4.62	4.68	**4.80**
Mean	**5.48**	**4.91**	**5.11**	**5.04**	
CD (5%)	Genotypes (G) = 0.04, Treatments (T) = 0.09, G X T = 0.10				
			Post-flowering stage		
Control	3.00	2.69	2.91	2.86	**2.87**
E_1V	4.04	3.86	3.92	3.89	**3.93**
E_2V	3.97	3.83	3.91	3.85	**3.89**
C_1V	3.94	3.81	3.90	3.84	**3.87**
C_2V	3.76	3.59	3.71	3.68	**3.69**
E_1F	3.91	3.79	3.89	3.82	**3.85**
E_2F	3.81	3.68	3.79	3.76	**3.76**
C_1F	4.00	3.86	3.93	3.88	**3.92**
C_2F	3.88	3.80	3.86	3.81	**3.84**
Mean	**3.81**	**3.66**	**3.76**	**3.71**	
CD (5%)	Genotypes (G) = 0.07, Treatments (T) = 0.08, G X T = 0.09				

vi) Relationship between photosynthetic rate and leaf traits

Photosynthetic rate is determined to some extent by sink growth rates in relation to leaf area (Del-Campo *et al* 2002), specific leaf weight and chlorophyll content. From the relationship between SLW and chlorophyll content with photostnthesis (Fig 19), it was clear that 87 % variation in rate of photosynthesis could be explained by variations in chlorophyll content and 67% variability in photosynthetic rate could be explained by variability in SLW. SLW and chlorophyll content are important measures for yield and photosynthesis. They are often used as selection indices for high yield probably due to their easily measurements.

Similar type of relationship between SLW and chlorophyll content was reported by Liu *et al* (2012).



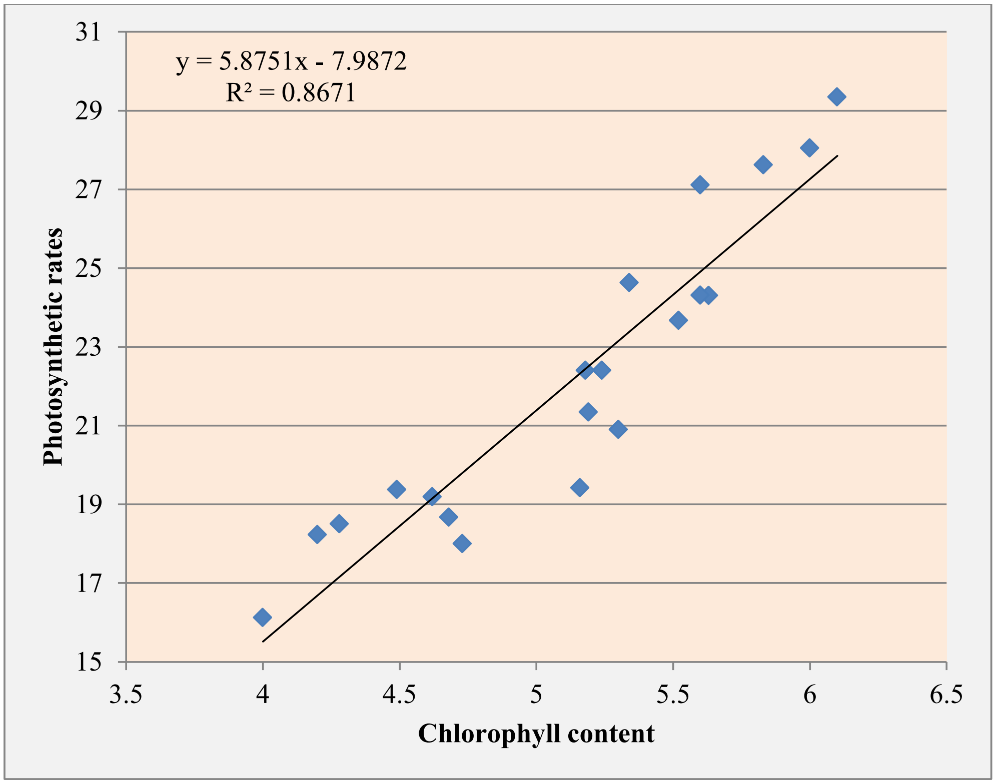


Fig. 19: Effect of SLW and chlorophyll content at flowering on photosynthetic rate

4.2.3 Phenological traits, number of flowers and pods/plant

i) Days to 50% flowering

The number of days to 50% flowering as influenced by different treatments showed significant differences (Table 14). In general, ethrel treatment took lesser days to 50% flowering as compared to those with other treatments. E_1V and E_2V treatment differed non-significantly as the plants with these treatments took 89 days to achieve 50% flowering followed by C_1V (91). However, in all genotypes number of days to 50% flowering were significantly more in control. The genotype AL 1578 took least number of days to 50% flowering (92) which wasat par with check variety AL 201.

ii) Number of flowers/plant

Yield in pigeonpea is mainly influenced by number of flowers produced/retained and the percent fruits developed with normal seeds. The plant produces a large number of flowers but all of them do not develop into pods. A great percentage of flowers abscise.

Various treatments differed non-significantly in producing number of flowers. However, there was a significant difference among genotypes. On mean basis, treatment E_2V produced maximum number of flowers/plant (Fig. 20). Among genotypes, AL 1578 produced maximum number of flowers (919.11) followed by AL 1702 (891.44). Genotype AL 1578 showed 10.29% increase in number of flowers as compared to check AL 201. Ethrel induced increase in flowering has been reported in mango (Thanda 2007) and chickpea (Saxena *et al* 2007). Treatments do not affect number of flowers/plant but help in retaining of flowers for effective pod formation.

iii) Number of pods/plant

The number of pods in untreated control plants ranged from 159-189. The plant growth regulators significantly influenced the number of pods per plant (Fig. 20). Both E_1V and E_2V treatments were more effective in enhancing number of pods per plant as compared to E_1F and E_2F treatment while lower concentration of its antagonist was more effective at flowering stage (i.e C_1F). Treatment E_1V and E_2V caused 23.16 and 22.88% increase in number of pods per plant while E_1F and E_2F caused only 15.54 and 10.87 % increase over control. C_1F caused 18.93% increase in number of pods per plant.

Genotype AL 1578 produced maximum (241) pods per plant under treatment E_2V followed by AL 1702 (203). Genotype AL 1578 produced 12.04 % (mean of all treatments) higher pods as compared to local check AL 201.

Due to indeterminate growth habit of pigeonpea there is insufficient availability of assimilates for the developing pods during reproductive phase, which sets in competition for nutrients among simultaneously growing stem, developing fruits and newly formed flowers. The significant increase in number of pods per plant in response to plant growth regulators

Table 14: Effect of ethephon and cobalt chloride on days to 50% flowering in four *Cajanus cajan* genotypes

Genotypes	**Treatments**									
	Control	**E_1V**	**E_2V**	**C_1V**	**C_2V**	**E_1F**	**E_2F**	**C_1F**	**C_2F**	**Mean**
AL 1578	95.00±1.00	89.36±2.64	89.00±4.00	90.30±3.00	94.50±2.50	93.30±3.30	94.20±1.84	93.20±1.20	92.80±1.80	**92.41±2.36**
AL 1593	96.30±2.30	90.12±1.88	90.30±3.70	91.80±3.20	96.00±2.00	94.40±2.40	95.00±1.88	94.00±1.00	94.20±2.20	**93.57±2.28**
AL 1702	96.00±1.00	90.00±1.00	89.76±1.24	92.00±2.00	95.30±2.20	94.20±2.84	95.20±2.20	94.00±2.00	95.00±2.00	**93.50±1.83**
AL 201	93.00±2.00	88.19±1.19	89.12±2.88	90.80±1.20	93.10±2.10	92.60±2.60	93.00±1.00	92.40±1.40	92.15±1.15	**91.60±1.72**
Mean	**95.08±1.58**	**89.42±1.68**	**89.54±2.96**	**91.22±2.35**	**94.73±2.20**	**93.63±2.79**	**94.35±1.73**	**93.40±1.40**	**93.54±1.79**	
CD (p=0.05)	Genotypes (G) = 0.008, Treatments (T) = 0.01, G X T = 0.02									

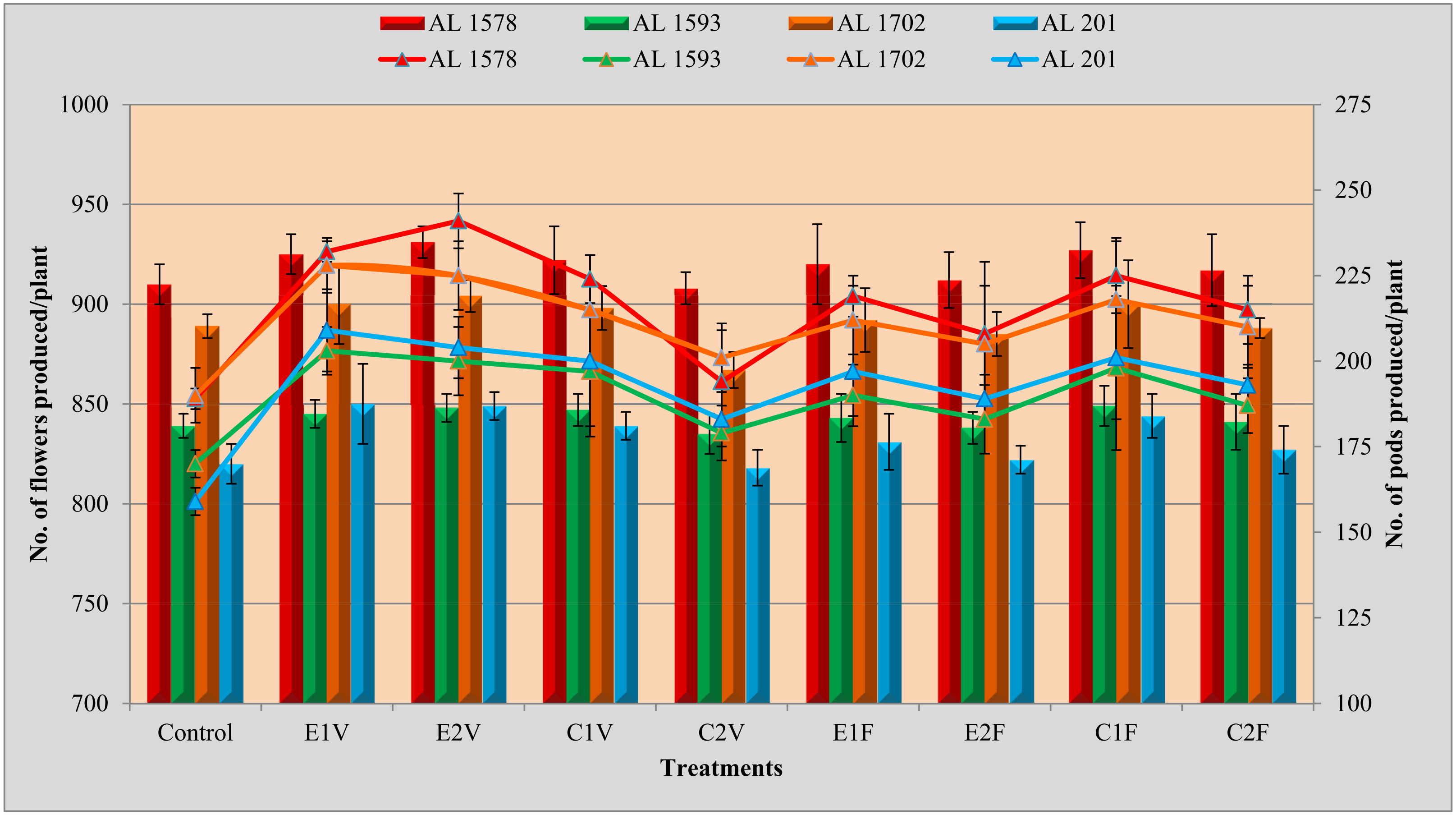


Fig. 20: Effect of foliar application of ethrel and cobalt chloride on number of flowers and pods/plant in pigeonpea genotypes

could have resulted due to enhanced supply of assimilates towards developing reproductive structures (Solaimalai *et al* 2001). However, the effect of PGRs is stage and concentration dependent. Ethrel was more effective at vegetative stage while lower concentration of $CoCl_2$ was more effective at flowering stage. Similar increase in number of pods per plant have been observed in chickpea with ethephon (Lone 2001, Saxena *et al* 2007) and in groundnut with cobalt (Basu *et al* 2006). The results suggest that for full effect of ethrel, spray should be done at early stage so as to provide sufficient time for the metabolic processes to result in morphological responses. Kandil (2007) also reported increase in number of pods/plant by lower concentration of cobalt in faba bean.

4.2.4 Partitioning

Partitioning of dry matter at different growth stages is presented in Tables 15, 16, 17, 18, 19 and 20. Total dry matter (shoot+leaf) at vegetative stage was highest (23.62g) in check genotype AL 201 followed by AL 1702 (22.58g), while genotype AL 1593 produced 17.95% less dry matter than local check at that stage. Partitioning of dry matter differs significantly in all genotypes. Genotype AL 1578 had highest leaf partitioning coefficient (63.63 %), which was 5.99% higher than local check but stem partitioning coefficient in this genotype was lowest amongst all genotypes.

At flowering stage, the total dry weight per plant increased with time from pre-flowering to flowering stage in control and treated plants, however, the magnitude of increase was higher in treated plants (Table 15). The shoot dry matter increased significantly over control, highest with E_1V treatment (72.21g/plant) followed by E_2V treatment (71.00g/plant). Leaf and flower dry matter also showed similar trend. Among the genotypes, AL 1578 showed maximum dry matter accumulation as compared to all other genotypes.

At flowering stage, partitioning of the dry matter towards shoot, leaves and flowers varied considerably with all treatments (Table 16). Partitioning of dry matter towards leaves was more in treated plants as compared to control plants and maximum partitioning coefficient was recorded with E_1V (33.92) followed by E_2V (33.53) and C_1V (33.21). However, in the shoot, with all treatments, dry matter partitioning coefficient was lower as compared to control. E_1V showed 4.89 % decrease in shoot partitioning coefficient as compared to control. Among the genotypes AL 1593 showed maximum (65.88 %) shoot partitioning coefficient followed by AL 1578 (65.14 %). In the flowers, control plants showed 1.65% coefficient of partitioning which was at par with C_2V (1.64 %). E_1V and E_2V treatment showed 2.09 and 1.94 % partitioning coefficient which was 26.66 and 17.58% higher than that of control. Among the genotypes, AL 1578 showed maximum coefficient of partitioning (1.95) in flowers.

Table 15: Effect of ethrel and cobalt chloride on dry matter (g) at flowering stage in pigeonpea genotypes

Genotypes	Treatments					
	E_1V	E_2V	C_1V	C_2V	Control	Mean
			Shoot dry matter			
AL 1578	74.67±2.37	74.20±4.80	71.80±2.20	68.17±3.17	69.88±4.77	**71.74±3.46**
AL 1593	68.89±8.89	67.20±3.80	65.36±3.64	67.64±4.64	66.48±6.36	**67.11±5.47**
AL 1702	74.47±4.47	72.60±3.40	69.70±3.30	67.89±3.89	65.99±2.99	**70.13±3.61**
AL 201	70.80±2.50	70.00±2.00	70.67±2.33	66.71±1.71	64.40±4.40	**68.52±2.59**
Mean	**72.21±4.56**	**71.00±3.50**	**69.38±2.87**	**67.60±3.35**	**66.69±4.63**	
			Leaf dry matter			
AL 1578	40.20±1.90	38.74±1.62	36.10±1.90	34.10±2.10	32.52±2.52	**36.33±2.008**
AL 1593	36.10±4.90	34.20±2.80	33.60±2.40	31.8±2.80	29.50±2.50	**33.04±3.08**
AL 1702	39.21±3.52	38.20±2.00	36.10±1.90	34.40±2.40	32.10±2.90	**36.00±2.54**
AL 201	37.64±3.02	36.51±2.39	36.00±2.00	33.31±2.31	30.00±2.00	**34.69±2.34**
Mean	**38.29±3.33**	**36.91±2.20**	**35.45±2.05**	**33.40±2.40**	**31.03±2.48**	
			Flower dry matter			
AL 1578	2.73±0.08	2.36±0.06	2.06±0.06	1.83±0.04	1.82±0.09	**2.16±0.07**
AL 1593	2.08±0.08	1.88±0.04	1.76±0.13	1.57±0.06	1.58±0.17	**1.77±0.10**
AL 1702	2.38±0.24	2.20±0.20	1.96±0.04	1.67±0.05	1.61±0.08	**1.96±0.12**
AL 201	2.26±0.06	2.11±0.21	1.96±0.14	1.66±0.06	1.56±0.11	**1.91±0.12**
Mean	**2.36±0.12**	**2.14±0.13**	**1.94±0.09**	**1.68±0.05**	**1.64±0.11**	

Table 16: Effect of ethrel and cobalt chloride on partitioning coefficient at flowering stage in pigeonpea genotypes

Genotypes	Treatments					
	E_1V	E_2V	C_1V	C_2V	Control	Mean
			Shoot partitioning			
AL 1578	63.50	64.35	65.30	65.49	67.05	**65.14**
AL 1593	64.34	65.06	64.89	66.96	68.14	**65.88**
AL 1702	64.17	64.25	64.68	65.30	66.19	**64.92**
AL 201	63.96	64.44	65.05	65.61	67.11	**65.23**
Mean	**63.99**	**64.53**	**64.98**	**65.84**	**67.12**	
			Leaf partitioning			
AL 1578	34.18	33.60	32.83	32.76	31.20	**32.91**
AL 1593	33.72	33.11	33.36	31.48	30.24	**32.38**
AL 1702	33.78	33.80	33.50	33.09	32.20	**33.27**
AL 201	34.00	33.61	33.14	32.76	31.26	**32.95**
Mean	**33.92**	**33.53**	**33.21**	**32.52**	**31.23**	
			Flower partitioning			
AL 1578	2.32	2.05	1.87	1.76	1.75	**1.95**
AL 1593	1.94	1.82	1.75	1.55	1.62	**1.74**
AL 1702	2.05	1.95	1.82	1.61	1.61	**1.81**
AL 201	2.04	1.94	1.80	1.63	1.63	**1.81**
Mean	**2.09**	**1.94**	**1.81**	**1.64**	**1.65**	

Table 17: Effect of ethrel and cobalt chloride on dry matter at post flowering stage in pigeonpea genotypes

Genotypes	Treatments									
	E_1V	E_2V	C_1V	C_2V	E_1F	E_2F	C_1F	C_2F	Control	Mean
					Shoot dry matter					
AL 1578	134.00±4.0	129.50±10.5	127.76±7.8	126.29±12.7	127.38±4.6	124.20±4.8	128.30±4.7	127.27±4.7	121.20±7.8	**127.32±6.9**
AL 1593	119.80±5.2	117. 50±7.5	113.97±4.0	108.50±10.5	115.30±7.7	106.20±15.8	113.80±5.2	113.20±3.8	101.70±10.3	**112.22±7.8**
AL 1702	128.00±6.0	123.10±7.9	121.8±4.2	119.20±9.8	121.40±7.6	119.94±6.1	121.30±4.7	121.37±2.6	117.20±8.8	**121.48±6.4**
AL 201	122.3±1.7	118.90±6.1	119.53±9.5	113.50±7.5	119.10±8.1	116.20±5.8	118.16±4.8	116.8±4.2	108.80±10.2	**117.03±6.4**
mean	**126.03±4.2**	**122.25±8.00**	**120.77±6.4**	**116.87±10.1**	**120.80±7.0**	**116.64±8.1**	**120.39±4.9**	**119.66±3.8**	**112.23±9.3**	
					Leaf dry matter					
AL 1578	22.83±2.8	23.26±1.7	20.50±2.2	18.60±1.6	20.38±1.4	19.94±0.3	21.79±4.2	20.08±1.8	18.30±1.3	**20.63±1.9**
AL 1593	17.52±1.4	17.80±2.2	16.90±1.6	15.10±0.2	16.50±2.2	15.40±1.9	17.40±1.6	16.10±1.1	14.90±1.0	**16.40±1.5**
AL 1702	23.26±1.9	21.46±1.5	20.46±2.5	17.30±0.3	19.77±2.4	17.80±0.8	21.39±2.6	19.60±1.6	16.70±2.4	**19.75±1.8**
AL 201	18.96±1.5	19.20±2.8	18.30±0.3	15.80±1.4	17.50±3.2	16.30±1.4	18.60±1.4	16.77±0.7	15.80±1.2	**17.47±1.5**
mean	**20.64±1.9**	**20.43±2.1**	**19.04±1.6**	**16.7±0.9**	**18.54±2.3**	**17.36±1.1**	**19.79±2.5**	**18.14±1.3**	**16.43±1.5**	
					Pods dry matter					
AL 1578	42.92±2.6	39.84±2.2	36.40±3.4	31.93±1.1	36.13±2.1	333.97±2.0	39.46±3.5	31.80±1.8	28.60±3.4	**35.67±2.5**
AL 1593	34.44±3.2	32.18±3.8	28.48±2.5	19.53±2.5	25.94±4.6	23.67±2.0	29.86±3.9	22.67±1.3	19.60±1.4	**26.26±2.8**
AL 1702	40.06±1.9	38.96±2.0	33.68±2.7	28.77±1.2	31.83±2.2	31.61±2.4	34.49±3.5	30.09±2.2	27.80±2.2	**33.03±2.3**
AL 201	36.12±1.9	33.49±1.5	31.25±2.3	23.99±2.0	30.52±2.5	29.14±2.9	31.19±1.8	28.77±3.4	21.44±1.6	**29.54±2.2**
mean	**38.39±2.4**	**36.12±2.4**	**32.45±2.7**	**26.06±1.7**	**31.10±2.9**	**29.60±2.3**	**33.75±3.2**	**28.33±2.2**	**24.36±2.1**	

Table 18: Effect of ethrel and cobalt chloride on partitioning coefficient at post-flowering stage in pigeonpea genotypes

Genotypes	Treatments									
	E_1V	E_2V	C_1V	C_2V	E_1F	E_2F	C_1F	C_2F	Control	Mean
					Shoot partitioning					
AL 1578	67.08	67.24	69.19	71.42	69.27	69.73	67.69	71.04	72.1	**69.42**
AL 1593	69.75	70.16	71.52	75.81	73.09	73.10	70.66	74.49	74.67	**72.58**
AL 1702	66.9	67.08	69.23	72.12	70.17	70.82	68.46	70.95	72.48	**69.80**
AL 201	68.95	69.29	70.7	74.04	71.27	71.89	70.36	71.95	70.95	**71.04**
mean	**68.17**	**68.44**	**70.16**	**73.35**	**70.95**	**71.39**	**69.29**	**72.11**	**72.55**	
					Leaf partitioning					
AL 1578	11.43	12.08	11.10	10.52	11.08	11.19	11.50	11.21	10.89	**11.22**
AL 1593	10.20	10.63	10.60	10.55	10.46	10.60	10.80	10.59	10.94	**10.60**
AL 1702	12.16	11.69	11.63	10.47	11.43	10.51	12.07	11.46	10.33	**11.31**
AL 201	10.69	11.19	10.82	10.31	10.47	10.08	11.08	10.33	10.82	**10.64**
mean	**11.12**	**11.4**	**11.04**	**10.46**	**10.86**	**10.6**	**11.36**	**10.9**	**10.75**	
					Pods partitioning					
AL 1578	21.49	20.69	19.71	18.06	19.65	19.07	20.82	17.75	17.01	**19.36**
AL 1593	20.05	19.21	17.87	13.64	16.45	16.29	18.54	14.92	14.39	**16.82**
AL 1702	20.94	21.23	19.14	17.41	18.4	18.67	19.47	17.59	17.19	**18.89**
AL 201	20.36	19.52	18.48	15.65	18.26	18.03	18.57	17.72	14.68	**17.92**
mean	**20.71**	**20.16**	**18.8**	**16.19**	**18.19**	**18.02**	**19.35**	**17.00**	**15.82**	

Table 19: Effect of ethrel and cobalt chloride on dry matter at maturity in pigeonpea genotypes

Genotypes	Treatments									
	E_1V	E_2V	C_1V	C_2V	E_1F	E_2F	C_1F	C_2F	Control	Mean
	Shoot dry matter									
AL 1578	140.00±8.0	132.20±8.8	129.76±3.2	125.79±12.2	128.38±10.6	125.10±4.9	132.50±6.5	125.47±5.5	125.47±6.5	**129.41±7.4**
AL 1593	125.10±5.1	121.24±7.8	117.17±5.8	109.07±8.9	119.00±4.0	108.20±5.8	119.92±9.1	114.82±4.2	111.59±4.4	**116.23±6.1**
AL 1702	134.30±4.7	127.90±4.1	124.90±3.1	122.00±9.0	126.20±7.8	123.14±5.9	129.50±5.5	123.77±7.2	106.47±9.5	**124.24±6.3**
AL 201	127.40±4.6	122.20±5.8	122.33±6.7	117.10±4.9	123.80±6.2	119.20±8.8	125.76±5.2	119.32±1.7	115.69±7.3	**121.42±5.7**
Mean	**131.70±5.6**	**125.89±6.6**	**123.54±4.7**	**118.49±8.8**	**124.35±7.2**	**118.91±6.3**	**126.92±6.6**	**120.85±4.7**	**114.81±6.9**	
	Leaf dry matter									
AL 1578	4.20±0.2	3.70±0.2	3.90±0.2	3.20±0.2	3.80±0.2	3.50±0.4	3.80±0.4	3.40±0.2	3.00±0.1	**3.61±0.23**
AL 1593	3.60±0.3	3.56±0.5	3.30±0.2	3.00±0.2	3.20±0.2	2.90±0.5	3.50±0.2	3.10±0.1	2.80±0.1	**3.22±0.25**
AL 1702	3.80±0.3	3.80±0.2	3.70±0.1	3.40±0.3	3.50±0.2	3.20±0.6	3.60±0.2	3.10±0.2	2.80±0.1	**3.43±0.24**
AL 201	3.90±0.1	3.70±0.3	3.50±0.1	3.20±0.2	3.20±0.2	3.30±0.6	3.70±0.6	3.30±0.3	2.80±0.1	**3.40±0.27**
Mean	**3.88±0.2**	**3.69±0.3**	**3.60±0.1**	**3.20±0.2**	**3.43±0.2**	**3.23±0.5**	**3.65±0.3**	**3.23±0.2**	**2.85±0.1**	
	Pods dry matter									
AL 1578	83.43±3.4	89.00±7.0	80.40±5.4	69.20±4.4	78.32±3.3	70.06±9.0	82.97±3.0	72.04±2.9	68.49±4.5	**77.10±4.8**
AL 1593	75.64±4.4	69.27±4.3	64.20±4.2	59.32±4.7	62.74±2.7	59.83±4.2	67.06±3.1	60.10±3.1	58.90±3.1	**64.12±3.7**
AL 1702	88.42±7.2	81.65±3.7	74.68±5.7	65.90±2.1	72.33±5.7	66.12±4.9	79.73±7.7	70.08±5.9	64.69±4.3	**162.62±5.2**
AL 201	79.18±4.2	74.28±3.3	69.74±4.7	62.82±1.2	65.15±3.8	63.79±5.2	73.82±3.8	64.97±5.0	62.08±3.9	**68.43±3.9**
Mean	**81.67±4.8**	**78.55±4.6**	**72.26±5.0**	**64.31±3.1**	**69.64±3.9**	**64.95±5.8**	**75.90±4.4**	**66.80±4.3**	**63.54±4.0**	

Table 20: Effect of ethrel and cobalt chloride on partitioning of dry matter at maturity in pigeonpea genotypes

Genotypes	Treatments									
	E_1V	E_2V	C_1V	C_2V	E_1F	E_2F	C_1F	C_2F	Control	Mean
					Shoot partitioning					
AL 1578	61.5	58.78	60.62	63.47	60.99	62.97	60.43	62.45	63.62	**61.65**
AL 1593	61.22	62.47	63.45	63.77	64.35	63.3	62.96	64.5	64.39	**63.38**
AL 1702	59.29	59.95	61.44	63.77	62.47	63.98	60.85	62.84	61.2	**61.75**
AL 201	60.53	61.05	62.55	63.95	64.43	63.99	61.87	63.61	64.07	**62.89**
Mean	**60.64**	**60.56**	**62.01**	**63.74**	**63.06**	**63.56**	**61.53**	**63.35**	**63.32**	
					Leaf Partitioning					
AL 1578	1.85	1.65	1.82	1.61	1.81	1.76	1.73	1.69	1.53	**1.72**
AL 1593	1.76	1.83	1.79	1.74	1.73	1.7	1.84	1.74	1.62	**1.75**
AL 1702	1.68	1.78	1.82	1.78	1.73	1.66	1.69	1.57	1.61	**1.7**
AL 201	1.85	1.85	1.79	1.75	1.67	1.77	1.82	1.76	1.55	**1.76**
Mean	**1.79**	**1.78**	**1.81**	**1.72**	**1.735**	**1.7225**	**1.77**	**1.69**	**1.58**	
					Pods partitioning					
AL 1578	36.65	39.57	37.56	34.92	37.21	35.27	37.84	35.86	34.86	**36.64**
AL 1593	37.02	35.69	34.76	34.48	33.92	35	35.21	33.76	33.99	**34.87**
AL 1702	39.03	38.27	36.74	34.45	35.8	34.36	37.46	35.58	37.19	**36.54**
AL 201	37.62	37.11	35.66	34.31	33.91	34.24	36.31	34.63	34.38	**35.35**
Mean	**37.58**	**37.66**	**36.18**	**34.54**	**35.21**	**34.7175**	**36.71**	**34.96**	**35.11**	

At post-flowering stage, due to formation of pods, partitioning of dry matter increased towards reproductive portions. Total plant dry matter increased in all treatments along with control (Table 17). Leaf dry matter during this stage decreased in control plants as compared to previous stages (due to reduced number of leaves at this stage). E_1V treatment produce maximum dry matter in pods (38.39) which was 57.59% more than that of control. However, treatment C_2V and control were at par with each other. Among genotypes AL 1578 produced highest dry matter as compared to all other genotypes.

Partitioning of dry matter towards pods was maximum with E_1V (20.71) followed by E_2V (20.16) while treatment C_2V did not show significant increase over control (Table 18). Increased coefficient of partitioning in leaves was observed with all treatments, but maximum was observed with E_2V (11.40) followed by C_1F (11.36) and E_1V (11.12). However, treatment C_2V showed decrease in coefficient of partitioning as compared to control (10.75). In shoots all treatments except C_2V recorded decrease in partitioning. Minimum partitioning towards shoots was observed with E_1V (68.17) followed by E_2V (68.44) treated plants. Control and C_2F treated plants were at par with each other.

At the time of harvest, maximum biomass was accumulated in E_1V (217.25g) treated plants followed by E_2V (208.13) and C_1F (206.47g) (Table 19). These treatments showed 19.89, 14.86 and 13.95% increase in total dry matter over control. Partitioning of dry matter towards pods was maximum in E_2V (37.66) followed by E_1V (37.58) and C_1F (36.70) treatments. Although, treatments C_2V, E_2F and C_2F recorded lesser coefficient of partitioning than control but the absolute dry matter accumulated in pods was more than control due to higher absolute accumulation of TDM with these treatments. In shoots, partitioning coefficient decreased with all treatments (Table 20). E_1V treatment showed maximum decrease (4.24%) in coefficient of partitioning than control. In leaves, maximum dry matter got accumulated with E_1V treatment (3.88g) against control (2.85g). Similarly, partitioning coefficient in leaves increased with treatments as compared to control. Maximum increase was observed in E_1V (13.15%) treated plants.

Among the genotypes AL 1578 recorded maximum TDM accumulation than all other genotypes. Pod partitioning coefficient was maximum in AL 1578 (36.63) followed by AL 1702 (36.54). Shoot partitioning coefficient was maximum in AL 1593 (63.38) followed by AL 201 (62.89).

Reddy (2009) concored that increase in yield due to growth retardants could be attributed to an increase in partitioning of assimilates towards developing sink (pods). Similar results were reported in oilseed crops by Mir *et al* (2010). Singh and Bhavna (2012) reported that NAA and $CoCl_2$ significantly increased partitioning of dry matter to reproductive parts. Gad (2012) found that lower concentration of cobalt resulted in increased dry matter distribution towards reproductive parts.

4.2.5 Yield parameters

i) Pod weight/plant

Pod weight /plant showed significant differences among genotypes and treatments. Among treatments E_1V treatment showed maximum pod weight followed by E_2V and C_1F and C_1V (Fig. 21). Lowest pod weight was noticed in control and C_2V.

Among genotypes, AL 1578 showed maximum (on mean basis) pod weight/plant followed by AL 1702 . Genotype AL 1578 showed 12.67% increase in pod weight/plant over local check AL 201 while AL 1593 showed 6.30% decrease in pod weight/plant.

ii) Seed weight/plant

Seed weight/plant is an important parameter which contributes directly to seed yield/plot. Maximum seed weight/plant was produced by E_1V treated plants followed by E_2V, C_1F and lowest was found in control plants (Fig. 21). Genotype AL 1578 produced maximum seed weight/plant followed by AL 1702. AL 1578 produced 9.93% higher seed weight/plant than AL 201.

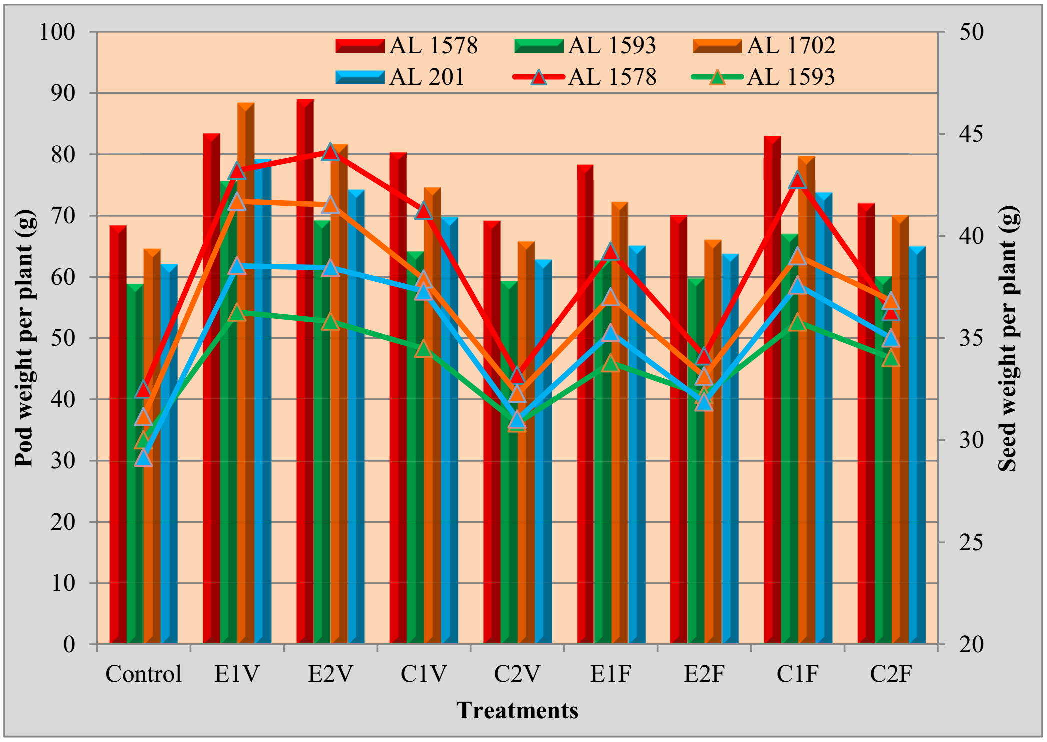


Fig. 21: Pod weight/plant and seed weight/plant in pigeonpea genotypes

iii) Pod set percentage

The data on pod set percentage presented in Fig. 22 indicated a significant increase due to treatments over control. Application of both concentrations of ethrel at vegetative as well as flowering stage resulted in better pod set as compared to control and showed 24.76 and 24.60 % pod set followed by C_1F (23.83%). Lowest pod set percentage was shown by control (20.45%) plants.

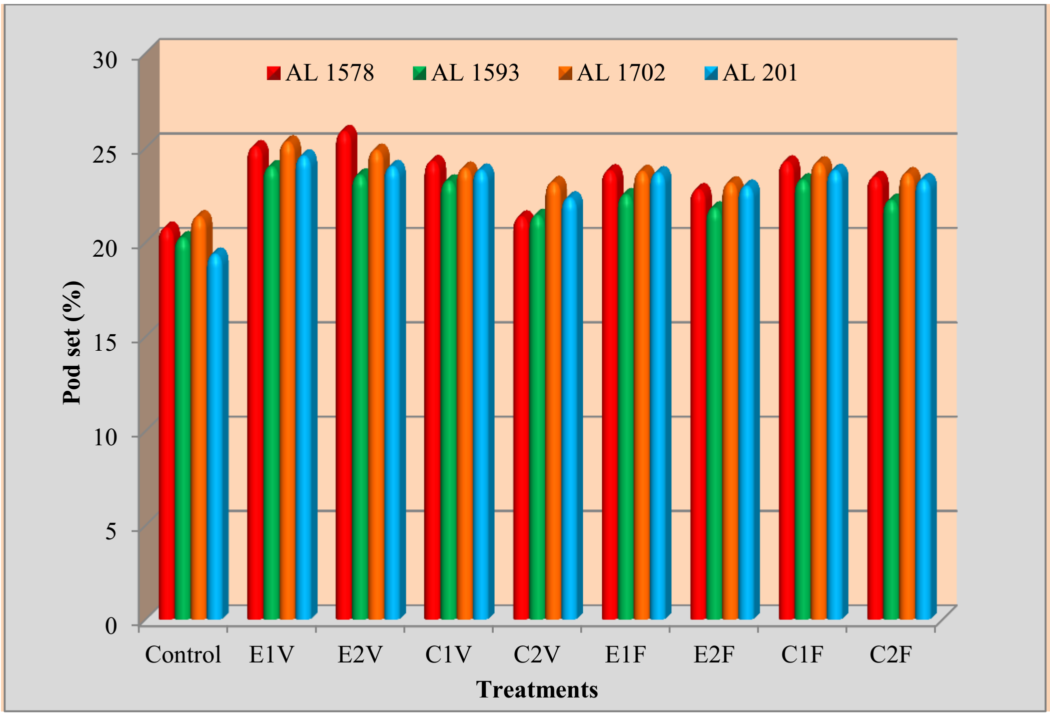


Fig. 22: Effect of ethrel and cobalt chloride on pod set percentage in pigeonpea genotypes

Among the genotypes, AL 1702 showed maximum (23.72%) pod set percentage followed by AL 1578 (23.52), while AL 1593 showed lowest pod set percentage (22.5%). The effect of ethrel unlike other PGRs viz. benzyladenine or triadimefon, primarily is not related to increase in number of reproductive units but their retention. The difference in this behavior can be attributed to the concentration and stage dependent effect on flower and pod retention. Our results are in agreement with Saxena *et al* (2000).

The reduction in flowers and pod abscission by E_1V, E_2V and C_1F treatment significantly increased the sink potential, as the reproductive components and pod set percentage were greater than control plants.

iv) 100-seed weight

The data on 100-seed weight revealed significant differences due to genotypes and growth regulator treatments (Fig 23). Treatment with ethrel recorded significantly higher 100-seed weight over its antagonist $CoCl_2$ and control. On mean basis, E_2V treated plants produced maximum 100-seed weight (7.48g) followed by C_1F (7.41g), E_1V (7.36g) and C_1V (7.27g). Lowest 100-seed weight was found in control plants. Among genotypes AL 201 produced maximum (7.29g) 100-seed weight followed by AL 1578 (7.23g).

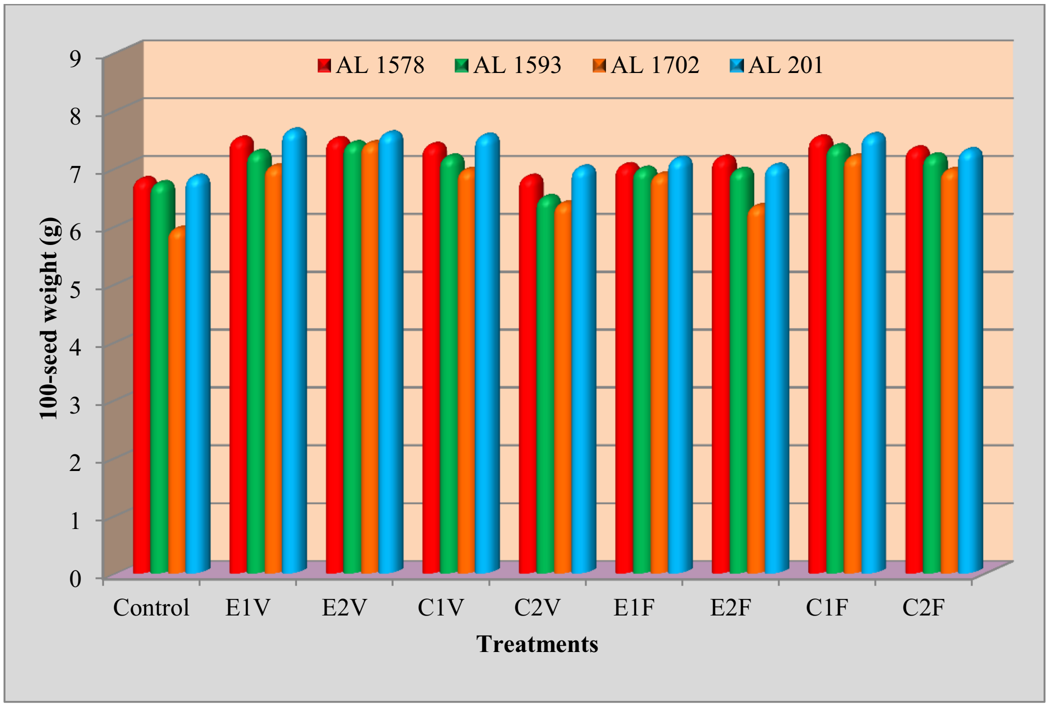


Fig. 23: Effect of foliar application of ethrel and cobalt chloride on 100-seed weight in pigeonpea genotypes

v) Seed yield (Kg/ha)

Seed yield was maximum in E_1V treatment followed by E_2V treatment and was found to be minimum in control plants (Fig 24). Among genotypes AL 1578 had maximum seed yield followed by AL 1702 and minimum seed yield was found in AL 1593.

vi) Harvest index (HI)

E_1V and E_2V treatments differed non-significantly from each other (Fig. 25). HI was found to be maximum in E_1V (16.54) treated plants. Lowest HI was found in control plants. AL 1578 had maximum HI with E_2V treatment (17.41) as compared to E_1V treatment. Among all genotypes AL 1593 had lowest HI.

Grain yield is the manifestation of morphological, physiological and biochemical parameters. Improvement of yield, according to Humphries (1979) could happen in two ways i.e., by adopting the existing varieties to grow better in their environment or by altering the relative proportion of different plant parts, so as to increase the yield of economically important parts. The increased seed yield could be due to higher dry matter production and its accumulation in reproductive parts. In the present investigation, it was observed that pod weight/plant, pod setting percentage, seed weight/plant, 100-seed weight, seed yield and harvest index increased due to plant growth regulator treatments. The increase in yield contributing parameters could probably be due to beneficial effects of PGRs, which are involved in enhancement of major physiological processes i.e., photosynthesis and nitrogen metabolism.

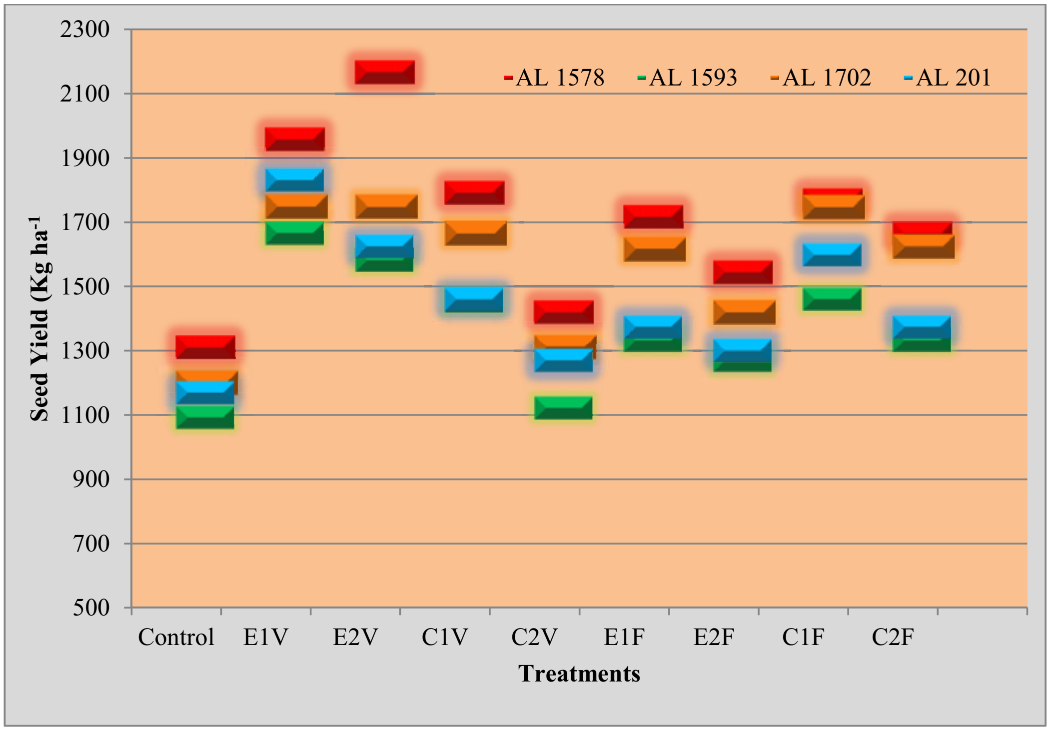


Fig. 24: Effect of foliar application of ethrel and cobalt chloride on yield in pigeonpea genotypes

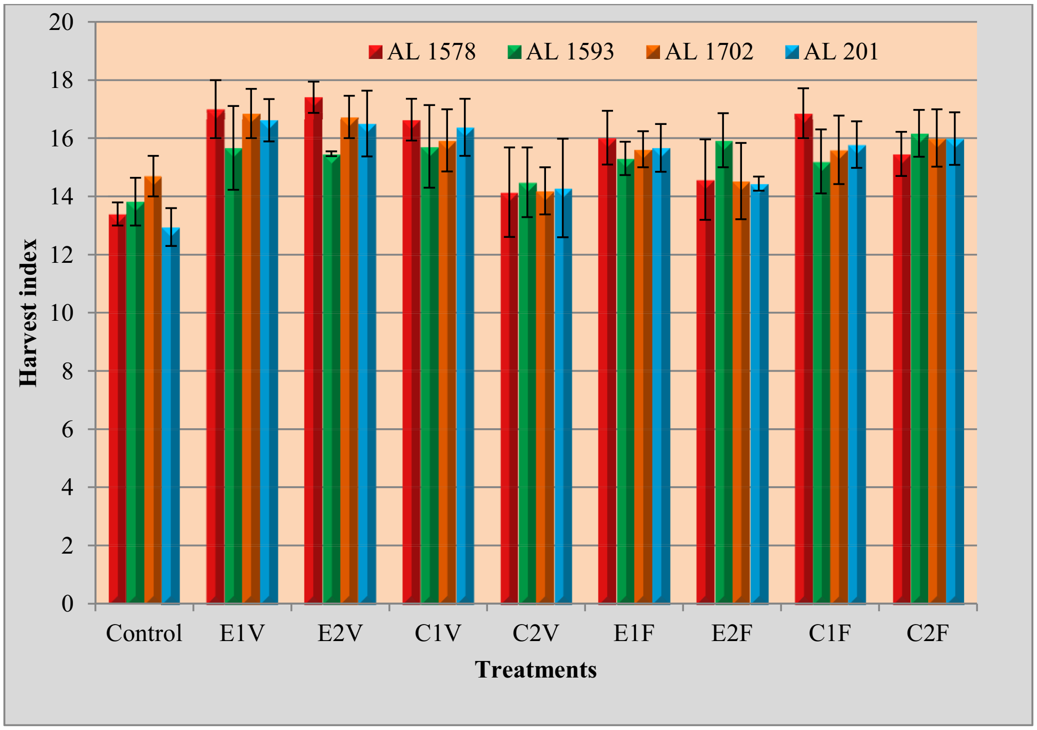


Fig. 25: Effect of foliar application of ethrel and cobalt chloride on harvest index in pigeonpea genotypes

Mir *et al* (2010) opined that lower concentration of ethrel improve yield of brassica. Devi *et al* (2011) reported that foliar application of ethrel exerted a significant effect on plant growth when applied at specific time. It increased the yield of soybean by increasing number of retained flowers, pods/plant and 100-seed weight by manipulating source-sink relationship. Bhat *et al* (2010) also reviewed that foliar application of ethrel improve yield contributing traits viz. number of pods/plant, seed number, 100-seed weight by manipulating source-sink relationship. Balai *et al* (2005) reported that lower concentration of cobalt increased dry matter accumulation, number of pods/plant as well as pod weight/plant. Similar results were obtained by Banerjee *et al* (2005). Kandil (2007) reported significantly higher number of pods/plant, number of seeds/plant, dry weight/plant and seed yield in faba bean due to application of lower concentration of cobalt. Saxena *et al* (2007) reported that increased seed yield with PGRs has been obtained due to earlier retention of more number of flowers and shift in balance of dry matter towards pods presumably favours the increased pod setting.

4.2.6 Biochemical analysis

The carbohydrates and proteins comprise important constituents of legume seeds. The relationship between the source (leaf) and Sinks (seeds) can be established based on the pattern of their accumulation in the two organs.

4.2.6.1 In leaves

i) Total soluble sugars

Total soluble sugars (TSS) contribute to the energy reserves and transportable carbohydrates generated during photosynthesis. Soluble sugars are the substrate for starch synthesis in plants. In general, TSS showed an increase from vegetative to post-flowering stage in all the genotypes. At vegetative stage, genotype AL 201 showed maximum TSS content (39.94 mg g^{-1} DW) followed by AL 1702 (38.3 mg g^{-1} DW) (Fig 26). Minimum TSS content in leaves was recorded in genotype AL 1593 (37.12 mg g^{-1} DW). At flowering stage, TSS increased as compared to vegetative stage. Treatment E_1V showed maximum increase in TSS (9.23%) as compared to control followed by E_2V (7.97%). C_2V and control differed non-significantly from each other. Among the genotypes, AL 1578 (on mean basis) showed maximum TSS (51.31 mg g^{-1} DW) followed by AL 201 (50.56 mg g^{-1} DW). Genotype AL 1578 showed maximum increase (8.38%) in TSS content under treatment E_2V followed by E_1V (6.88%) as compared to that of control. At post-flowering stage, similar trend was observed, i.e. TSS content was maximum in treated plants as compared to control. Maximum TSS were recorded in E_1V treatment followed by E_2V, C_1F and C_1V which showed 8.66, 5.89, 5.08 and 4.86 % increase than that of control. Among genotypes AL 1578 showed maximum (64.52 mg g^{-1} DW) TSS content which was 1.86 % higher than that of control.

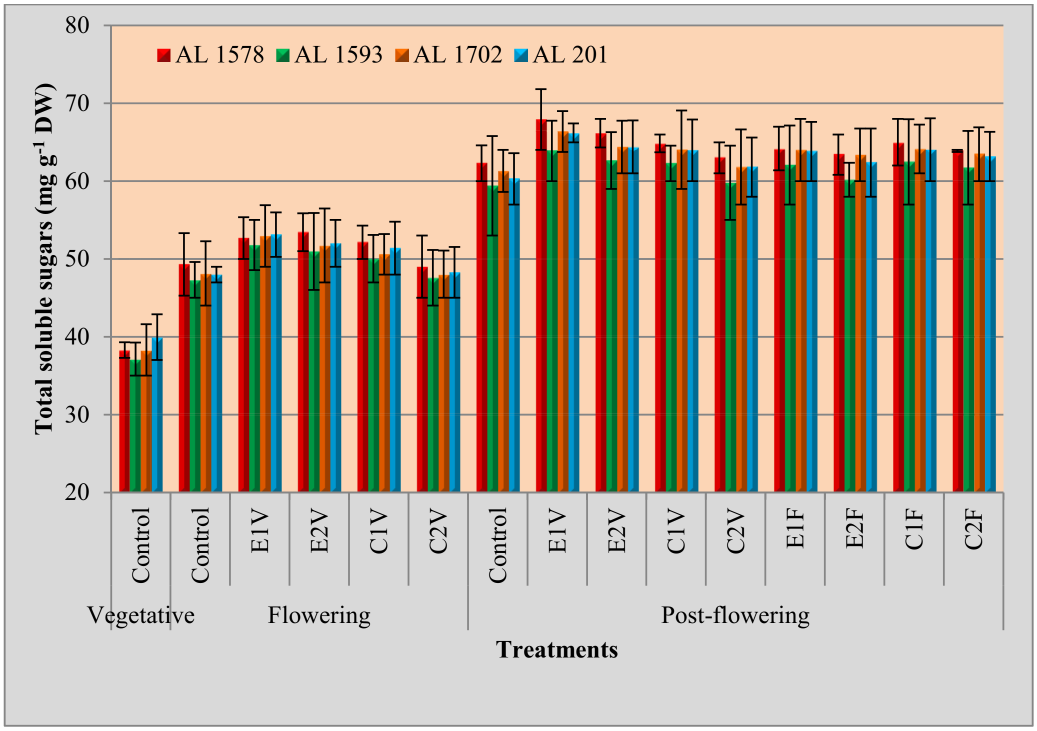


Fig. 26: Effect of foliar application of ethrel and cobalt chloride on total soluble sugars in pigeonpea genotypes

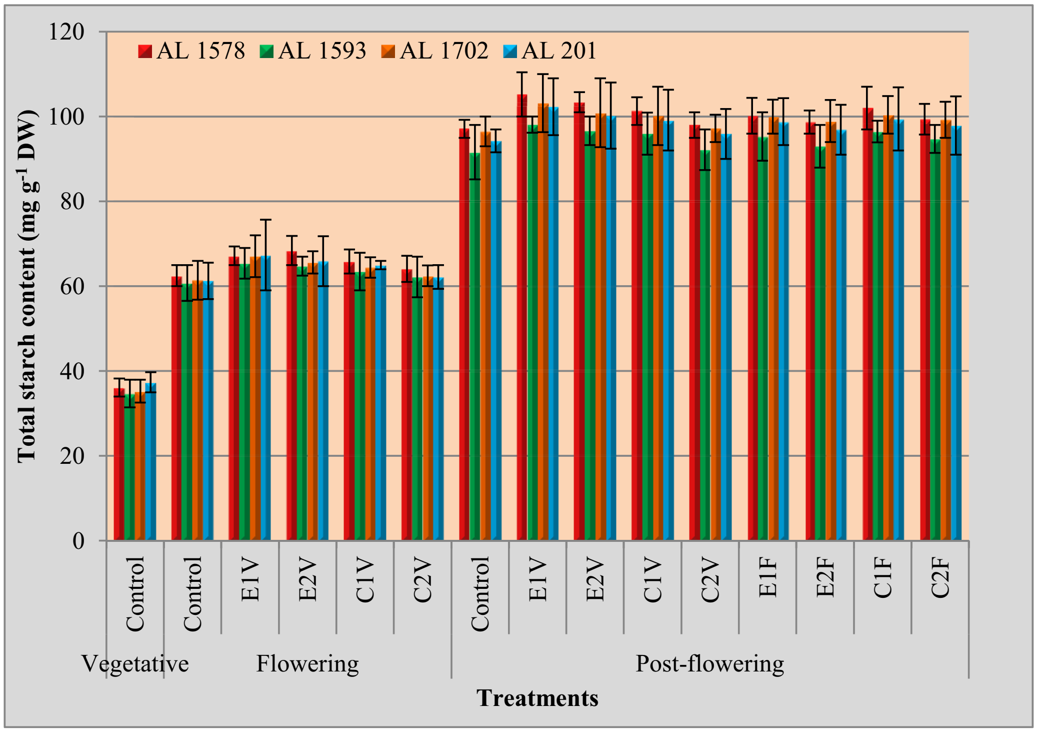


Fig. 27: Effect of ethrel and cobalt chloride on total starch content in leaves of pigeonpea genotypes

ii) Total starch content

Starch is the major storage form of carbohydrates. In general starch biosynthesis occurs in plastids of both photosynthetic and non-photosynthetic cells (Zeeman *et al* 2010). The starch content in leaves increased from vegetative to post-flowering stage in all pigeonpea genotypes with all treatments including control. E_1V treatment enhanced the leaf starch content at all stages of growth more prominently than all other treatments (Fig. 27).

At vegetative stage, check genotype AL 201 had maximum (37.38 mg g^{-1} DW) starch content which was 7.69% higher than AL 1593. At flowering stage, treatment E_1V recorded 66.74 mg g^{-1} DW starch content. Among genotypes higher starch content was shown by AL 1578 (65.61 mg g^{-1} DW) followed by AL 201 (64.34 mg g^{-1} DW). In genotype AL 1578, E_2V treatment was more (9.49%) capable of increasing starch content as compared to E_1V (7.50%) treatment. At post-flowering stage, E_1V treatment showed maximum (102.19 mg g^{-1} DW) starch content followed by E_2V (100.28 mg g^{-1} DW). Among the genotypes AL 1578 showed highest (100.58 mg g^{-1} DW) starch content which was 6, 2.38% higher than genotypes AL 1593 and AL 201 respectively.

The level of these biomolecules in these organs increased during development is indicative of high metabolic status. Wilcox (2001) indicated that the soluble sugar content plays a very important role in carbohydrate metabolism and has a close relationship with photosynthesis and production. The level of soluble sugar content was a sign of the supply ability of leaves and reflected transformation and ability of grains to use assimilates (Saratha *et al* 2001). Wang *et al* (2007) reported that the photosynthate exists as a form of water soluble carbohydrate and its main components are soluble sugars, starch and cellulose.

iii) Total free amino acids

The level of free amino acids also showed a continous increase from vegetative to post- flowering stage (Fig. 28). At vegetative stage, genotype AL 1702 recorded maximum free amino acids (7.58) followed by AL 1578 (7.43). At flowering stage E_1V and E_2V treatment (on mean basis) differs non-significantly from each other as they recorded 21.31 and 21.02 mg g^{-1} DW free amino acid content respectively. Among genotypes, AL 1578 recorded maximum amino acid content (20.97 mg g^{-1} DW) followed by AL 1702 (19.77 mg g^{-1} DW). At post flowering stage, E_1V and E_2V treatments (on mean basis) recorded 35 mg g^{-1} DW amino acids content followed by C_1F (34.52 mg g^{-1} DW), C_1V (34.20 mg g^{-1} DW) treatment. At this stage also, genotype AL 1578 showed highest (34.85 mg g^{-1} DW) amino acid content.

iv) Total soluble proteins

Total soluble proteins showed similar trend as that of amino acids i.e it increased from vegetative to post-flowering stage in leaves (Fig 29). At vegetative stage, total soluble

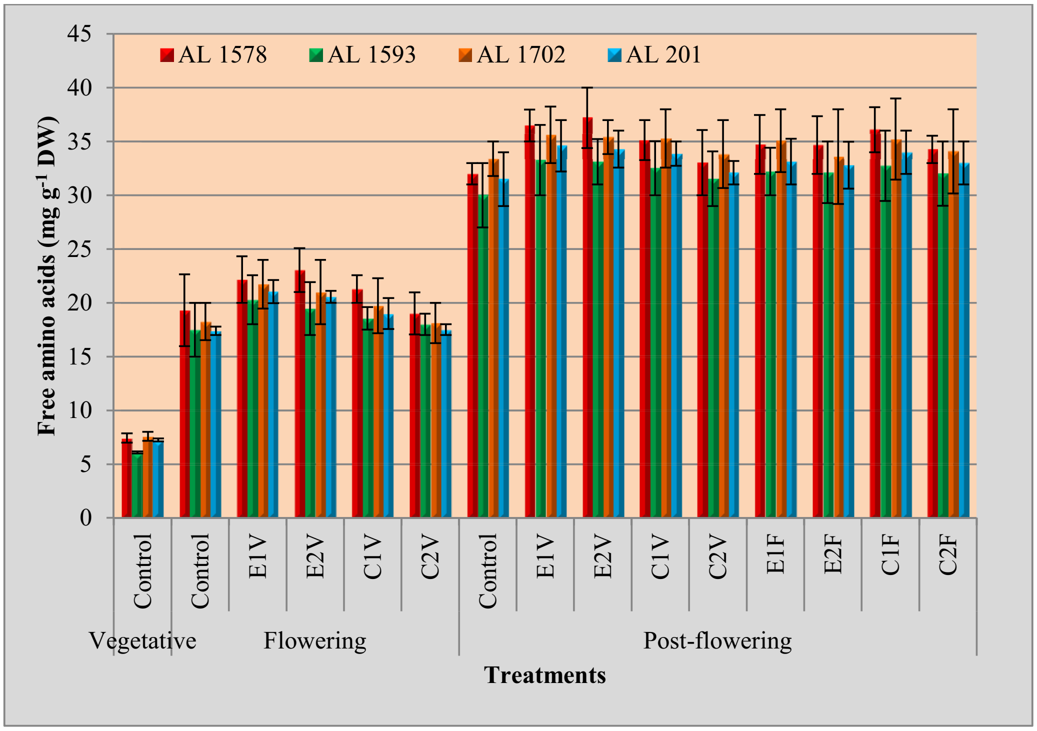


Fig. 28: Effect of foliar application of ethrel and cobalt chloride on free amino acids in leaves of pigeonpea genotypes

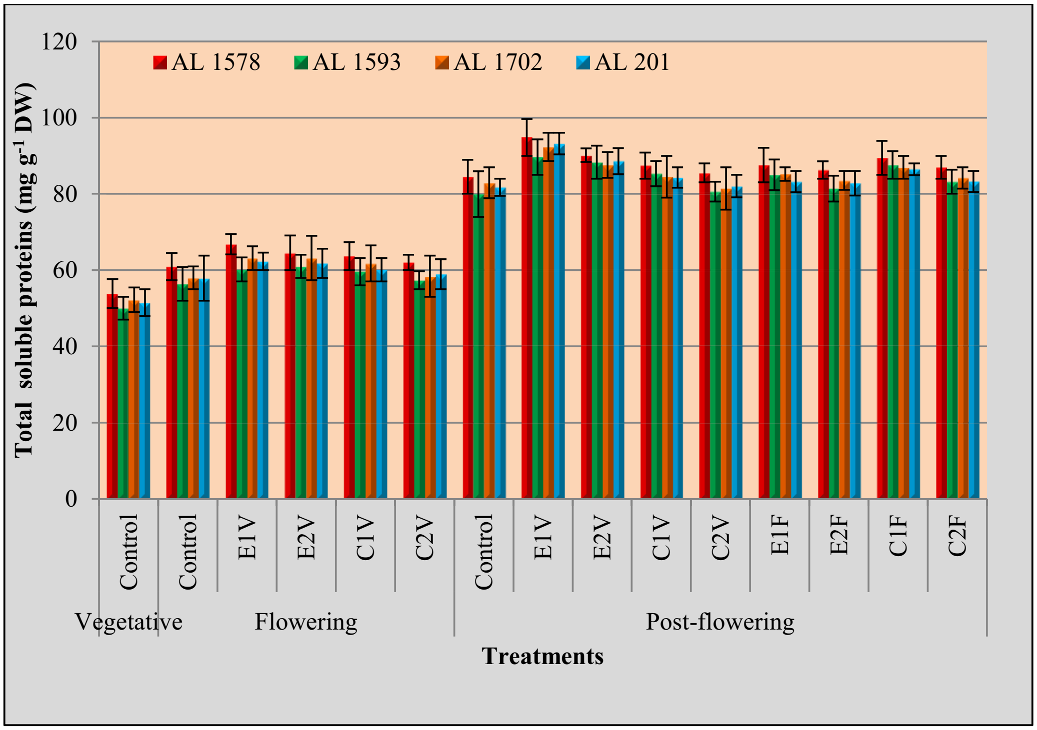


Fig. 29: Effect of foliar application of ethrel and cobalt chloride on total soluble proteins in leaves of pigeonpea genotypes

proteins ranged from 50-53.82 mg g^{-1} DW (Fig. 27). Genotype AL 1578 recorded maximum (53.82 mg g^{-1} DW) protein content as compared to all other genotypes. At flowering and post-flowering stage, E_1V treatment showed maximum proteins followed by E_2V and C_1F. At flowering stage E_1V treatment recorded 8.23% increase in protein content, while E_2V and C_1F showed 6.90 and 5.09% increase in protein content as compared to control. At post-flowering stage, E_1V showed 12.40% increase in protein content followed by E_2V (7.77%) treatment.

4.2.6.2 In seeds

i) Total soluble sugars (TSS)

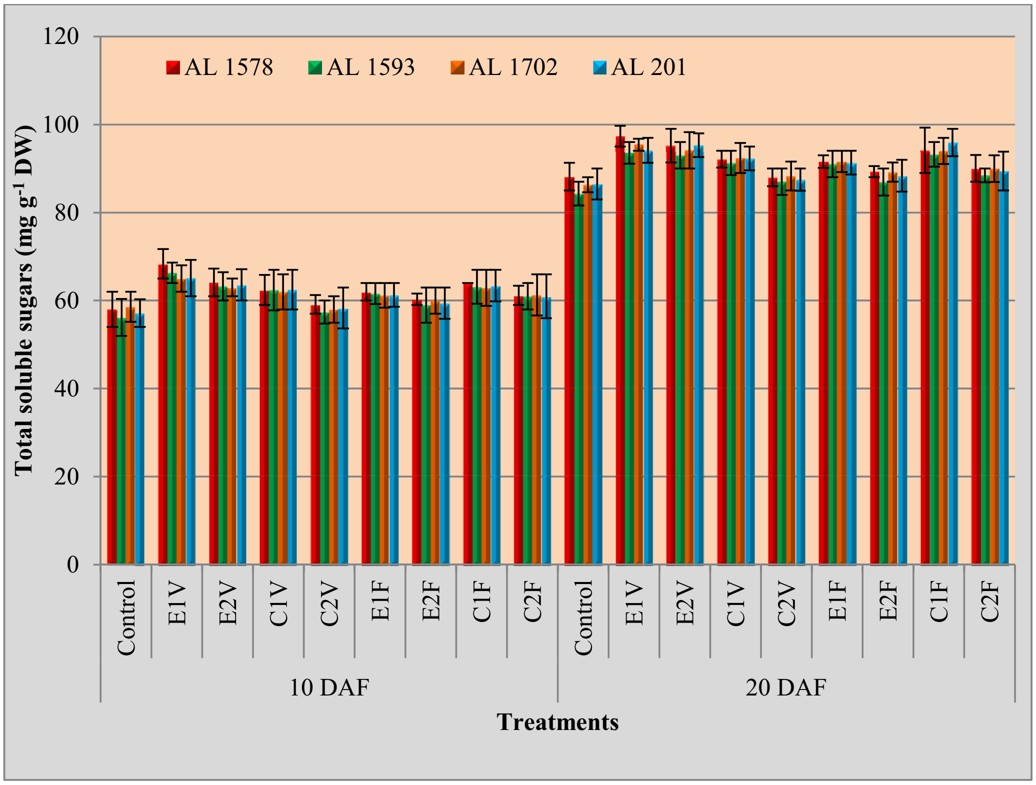
In developing seeds total soluble sugars first increased from 10 days after flowering (DAF) to 30 DAF and then decreased at maturity (Fig. 30). At 10 DAF lower concentration of ethrel (E_1V) followed by E_2V showed maximum TSS, which were 15.17 and 10.43 % higher than that of control. Similar trend was seen at 20 DAF. At 30 DAF and maturity, treatment E_1V followed by C_1F showed maximum TSS content (137.84, 135.12 mg g^{-1} DW, respectively, at 30 DAF and 102, 101.34 mg g^{-1} DW, respectively, at maturity) TSS than all other treatments.

ii) Total starch in seeds

In seeds, there was very high starch content at maturity (Fig. 31). At 10 DAF, C_2F treatment registered maximum (138.65 mg g^{-1} DW) total starch content in developing seeds followed by E_2F (138.65 mg g^{-1} DW). Lowest seed starch content was found in E_2V (126.66 mg g^{-1} DW). At 20 DAF, maximum starch content was found in C_1F treatment (182.62 mg g^{-1} DW) followed by E_2V (180.50 mg g^{-1} DW) and E_1V (179.94 mg g^{-1} DW) which showed 7.53, 6.29, 5.95 % increase than that of control. At 30 DAF, E_2V treatment (on mean basis) showed maximum (249.85 mg g^{-1} DW) starch content while at maturity E_1V (346.84 mg g^{-1} DW) followed by E_2V (343.13 mg g^{-1} DW) showed maximum starch content. Among genotypes, AL 1578 registered maximum starch content at all stages of seed development and lowest was found in AL 1593.

iii) Total free amino acids

The seed amino acid content decreased from 10 DAF till maturity (Fig. 32). At 10 DAF maximum free amino acids were recorded in E_2V and E_1V which had 43.32, 43.13 mg g^{-1} DW amino acid content in developing seeds. Lower amino acid content was recorded in control plants. At 20 DAF, E_1V treatment showed maximum (30.14 mg g^{-1} DW) amino acid content followed by C_1F (29.79 mg g^{-1} DW) which showed 11.30 and 10.01 % increase than that of control. At 30 DAF, E_2V showed 22.68 mg g^{-1} DW amino acid content followed by E_1V. However, at maturity, treatment E_1V registered maximum amino acid content (12.46 mg g^{-1} DW) followed by C_1F (12.21 mg g^{-1} DW).



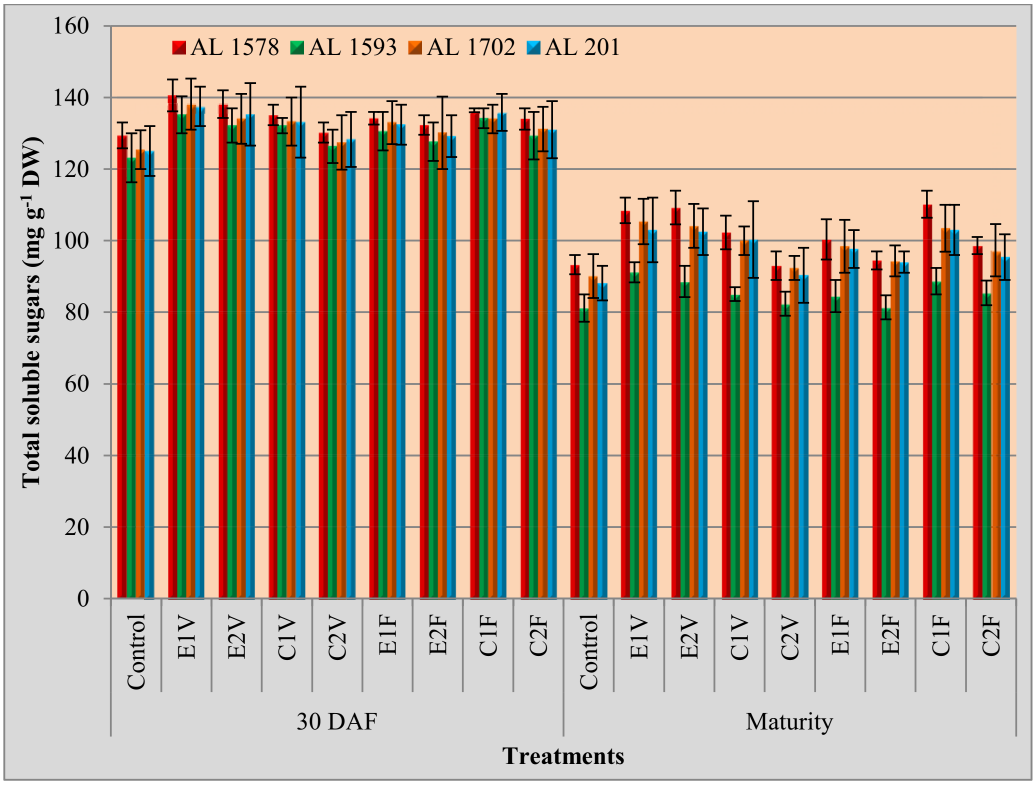
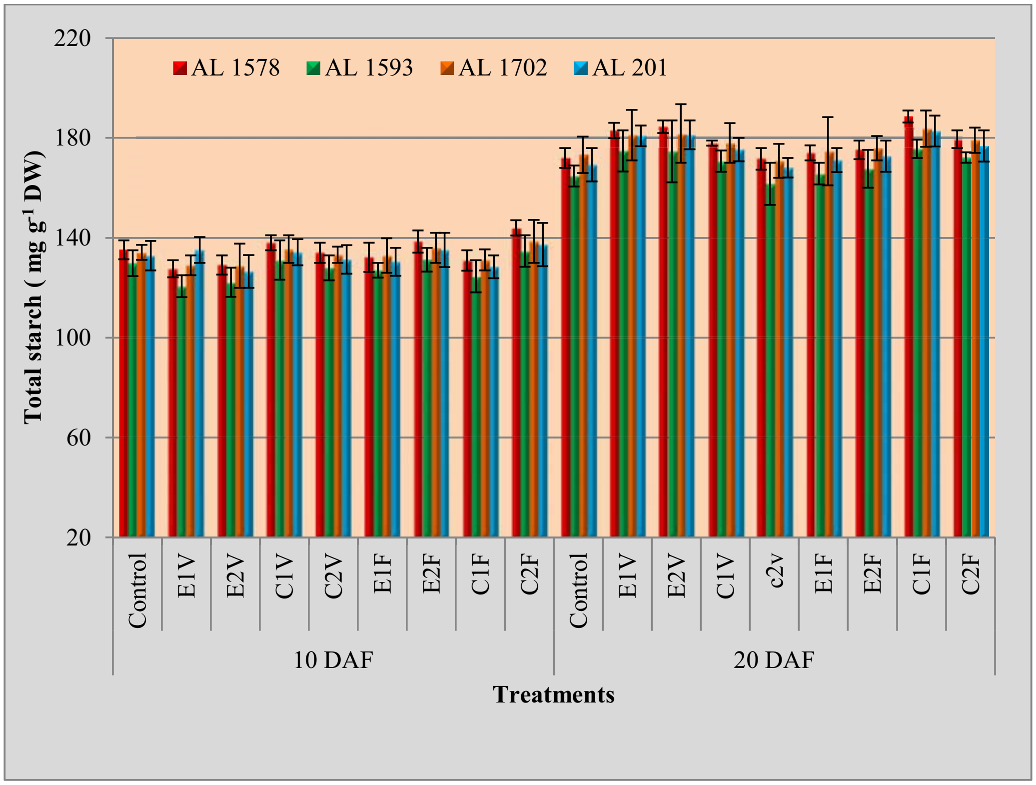


Fig. 30: Effect of ethrel and cobalt chloride on total soluble sugars in developing seeds of pigeonpea genotypes



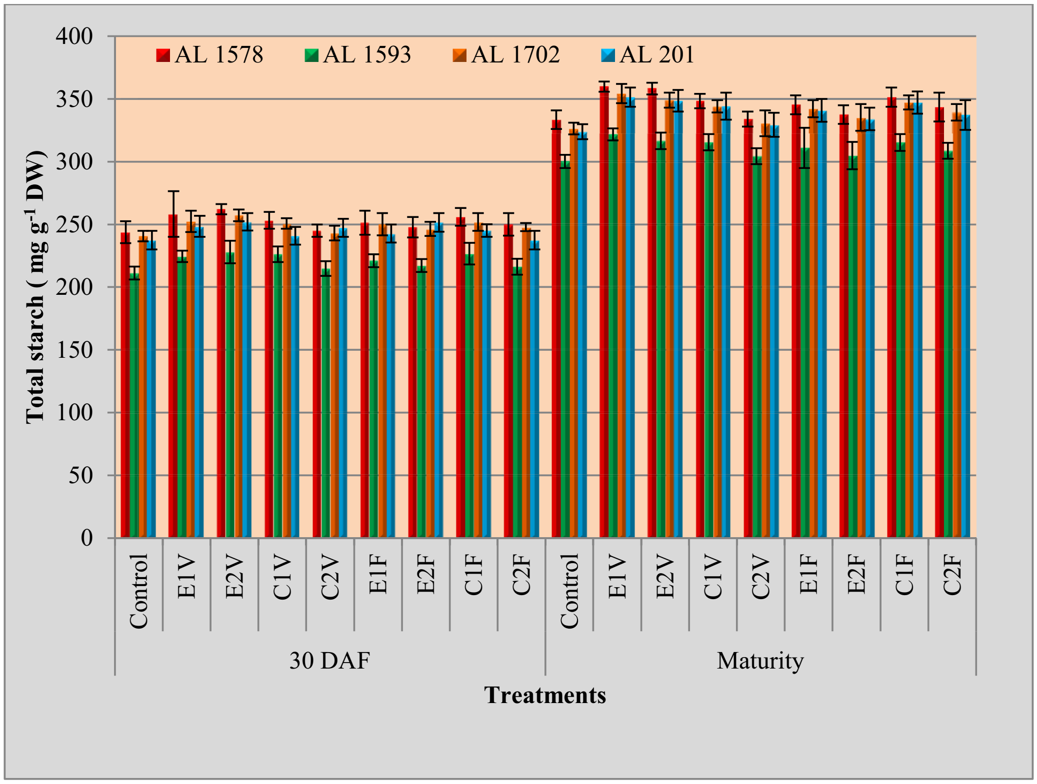
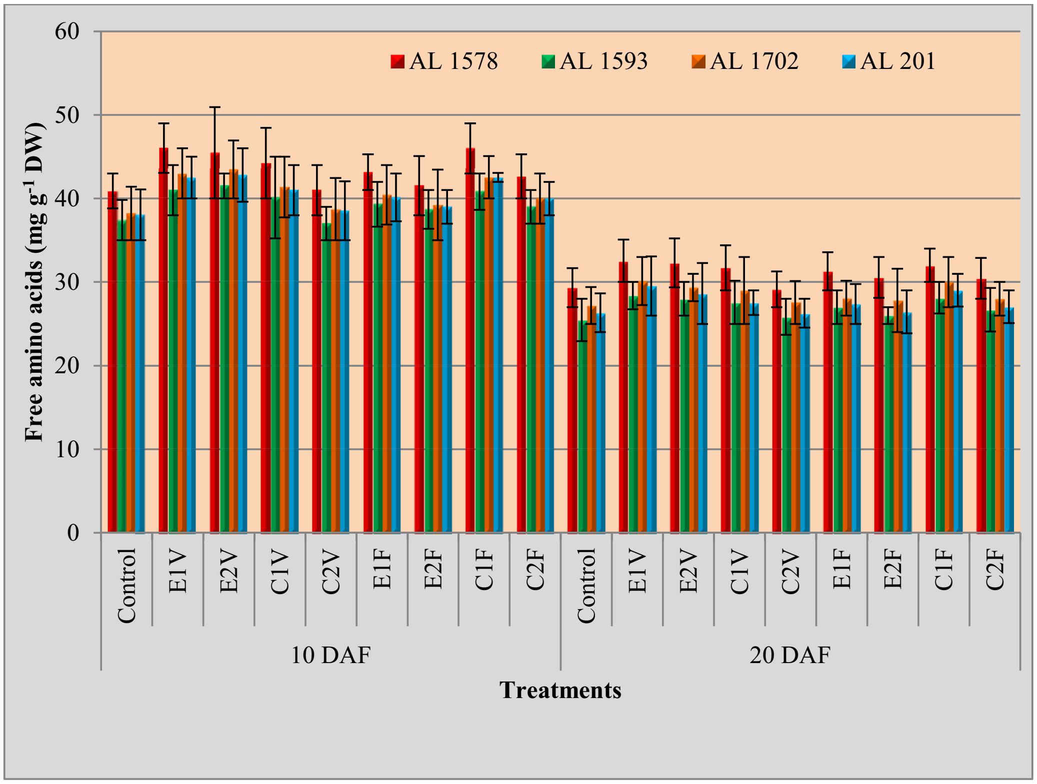


Fig. 31: Effect of ethrel and cobalt chloride on total starch content in developing seeds of pigeonpea genotypes



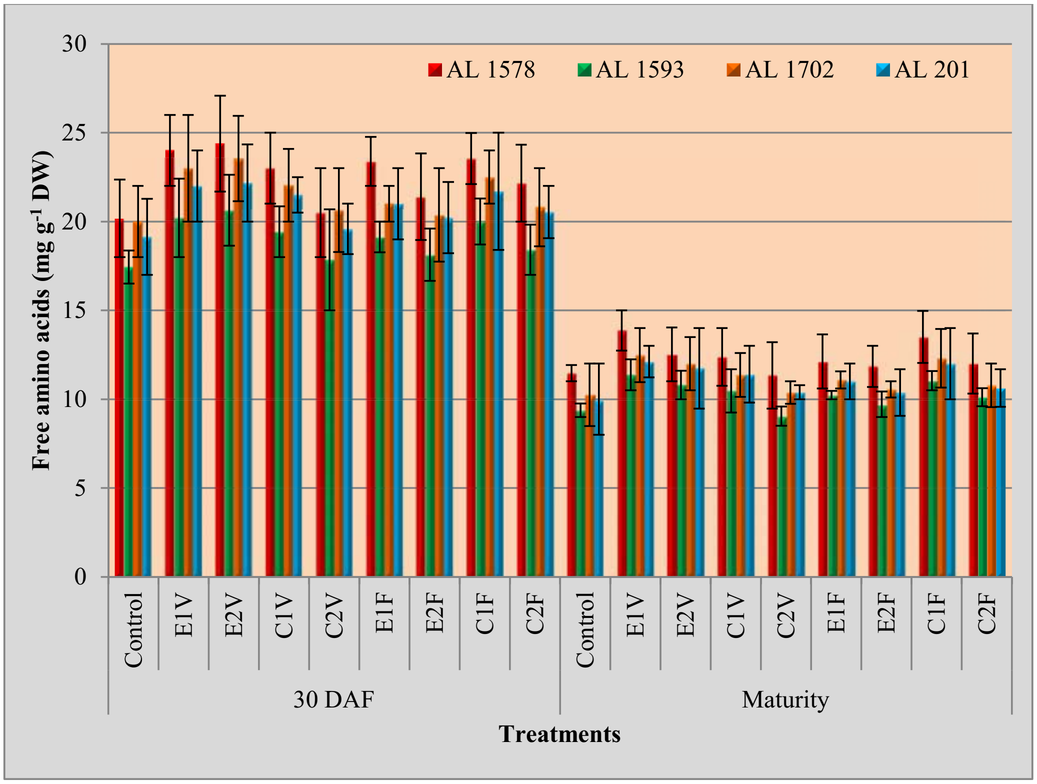
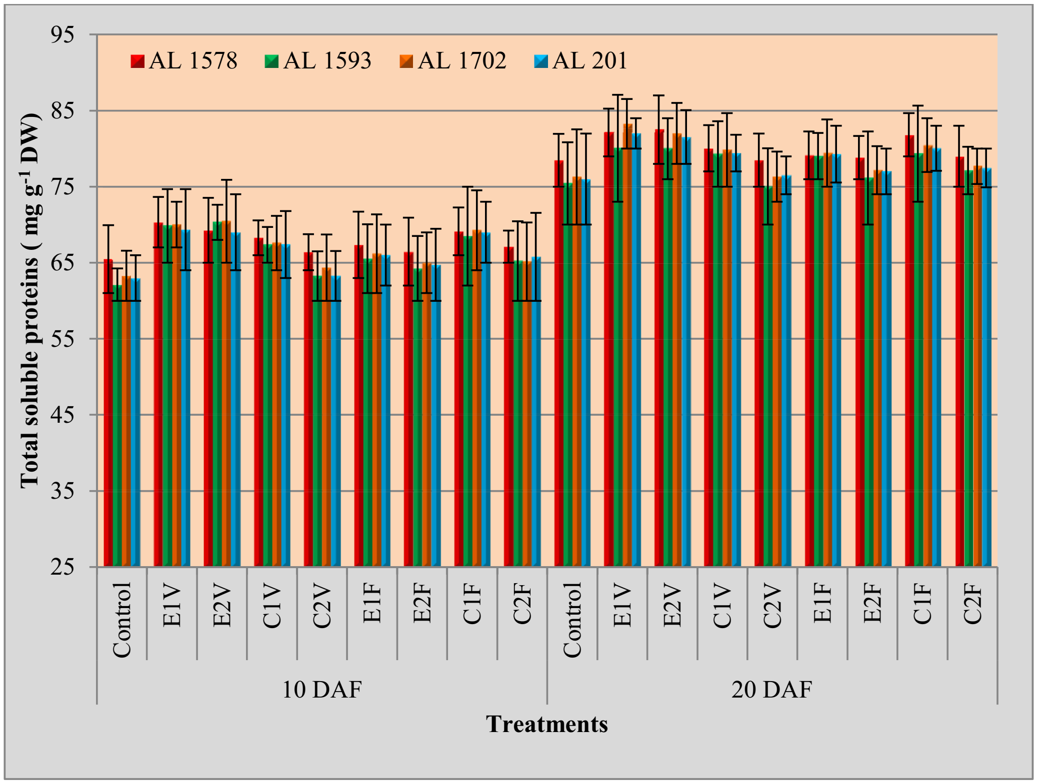


Fig. 32: Effect of ethrel and cobalt chloride on free amino acids in developing seeds of pigeonpea genotypes



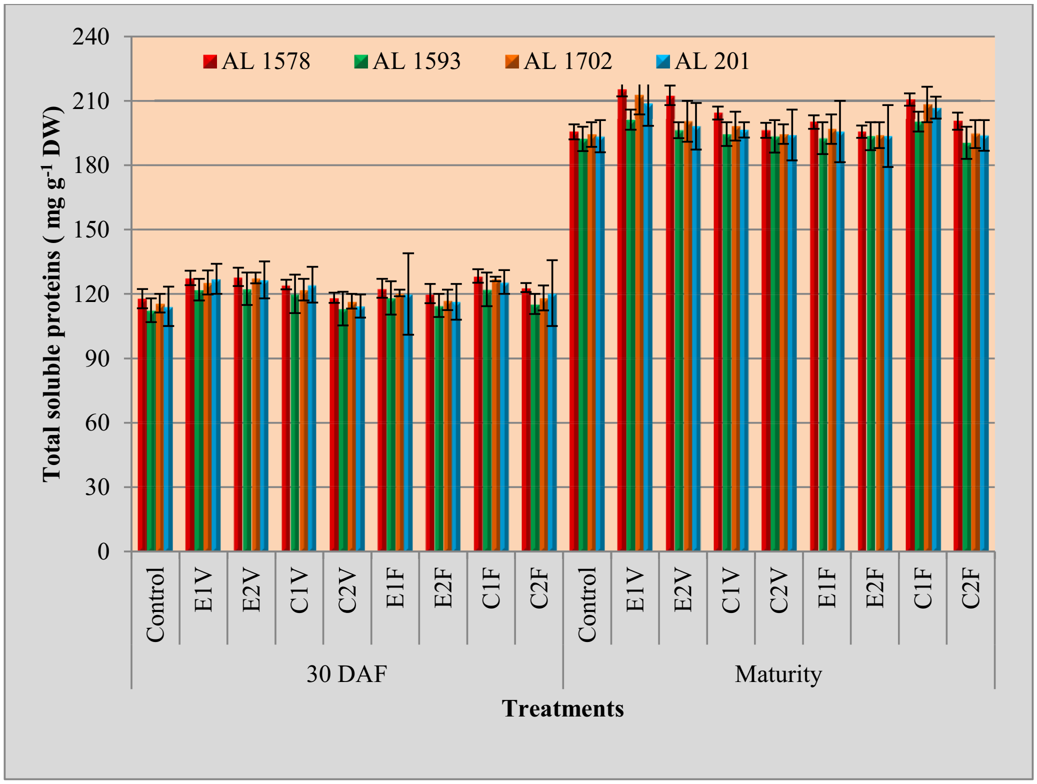


Fig. 33: Effect of ethrel and cobalt chloride on total soluble proteins in developing seeds of pigeonpea genotypes

iv) Total soluble proteins

Seed protein content increased continously from 10 DAF till maturity (Fig. 33). At 10 DAF, treatments E_1V and E_2V differed non-significantly (mean of all genotypes), they showed 69.88 and 69.76 mg g^{-1} DW protein content. Similar trend was seen at 20 DAF. At 30 DAF, E_2V treatment showed maximum (126.13 mg g^{-1} DW) protein content followed by C_1F (125.77 mg g^{-1} DW) treatment. At maturity, E_1V followed by C_1F showed 8.04 and 6.50 % increase in protein content over control. Maximum protein content was noticed in genotype AL 1578.

In the present study, it was observed that there was a significant increase in total sugars with the application of PGRs. The maximum TSS in leaves was recorded in E_1V, E_2V and C_1F treated plants. Similarly, Gad and Kandil (2008) showed that addition of 10 ppm cobalt had a synergistic effect on the sweet potato growth and increase in starch, sugars and carotenoids.

Gad *et al* (2013) reported that amount of TSS, total carbohydrates, total proteins and total phenols increased in *Ocimum basilicum* by application of lower concentration of cobalt. Gad *et al* (2011) reported an increase in protein and sugars following cobalt application at low concentration. Jaleel *et al* (2009) reported that low concentration of cobalt increases growth, biochemical constituents (total soluble sugars, starch and amino acids) and yield in *Zea mays.* Kandil (2007) reported an increased protein content by 47.82% in fababean following cobalt application.

Burstin *et al* (2007) reviewed that seed weight and protein content are mostly determined by the availability of assimilates (source strength) but variability in sink strength (assimilate demand) also plays an important role. Grain protein content is probably associated with their ability to accumulate higher amounts of nitrate in their leaves during vegetative growth and then to efficiently remobilize the stored nitrate during grain filling (Mickelson *et al* 2003). In developing seeds initial slow accumulation of these reserves then a sudden increase was observed during later stages of development. The levels of these reserves were higher in ethrel treated plants followed by plants treated with C_1F treatment.

4.2.6.3 Enzyme activity

i) Nitrate reductase activity in leaves

Nitrate reductase (NR) is the first enzyme in the inorganic nitrogen assimilatory pathway. It is a substrate-inducible enzyme and is the most limiting step in nitrogen assimilation. For this reason, nitrate reductase activity may be a selection criterion for grain yield and nitrogen assimilation potential (Kelly *et al* 1995). Legumes have always been a special target for the study of nitrogen metabolism, due to high protein content, their levels of nitrate reductase activity and formation of nodules where symbiosis with Rhizobium takes place. Nitrate reductase activity at different growth stages is presented in Fig. 34.

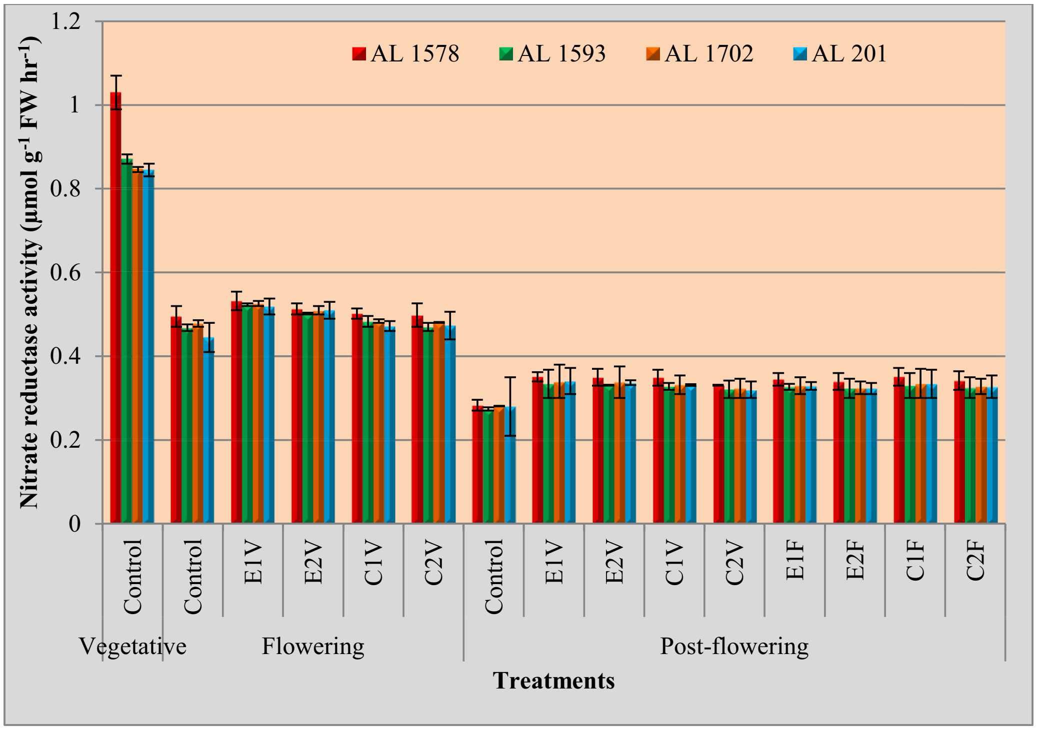


Fig. 34: Effect of foliar application of ethrel and cobalt chloride on nitrate reductase activity in leaves in pigeonpea genotypes

In general, the nitrate reductase activity was more at vegetative stage and it was reduced as the age of crop advanced (Fig. 30). Treatments with E_1V, E_2V, C_1F and C_1V registered high NR activity than that of control at all stages of plant growth. At vegetative stage, highest NR activity was recorded in AL 1578 (1.030 µmol g^{-1} FW hr^{-1}) followed by AL 1593 (0.871 µmol g^{-1} FW hr^{-1}) and lower in local check AL 201 (0.845 µmol g^{-1} FW hr^{-1}). At flowering stage, NR activity was maximum in E_1V (0.525 µmol g^{-1} FW hr^{-1}), which was 11.23% higher than that of control. Among the genotypes, AL 1578 followed by AL 1702 showed maximum (0.508 and 0.496 µmol g^{-1} FW hr^{-1} respectively) NR activity. At post-flowering stage, ethrel treatment at vegetative stage (E_1V and E_2V) significantly maintained higher level of nitrate reductase activity than that of control. Treatment E_1V showed 0.342 µmol g^{-1} FW hr^{-1} NR acitivity, which was 22.58 % higher than that of control. Genotype AL 1578 showed 4% more NR activity than that of check genotype AL 201.

The active accumulation of reduced nitrogen during vegetative and anthesis stage is an important factor that is associated with high yields (Swank *et al* 1982). Maximum leaf nitrate reductase activity, which represents the capacity of the plant to reduce inorganic nitrogen (Masclaux *et al* 2000) was maximum in treated plants as compared to control. Similar results were also found by Reddy *et al* (2009). Nitrate reductase activity increased by application of lower concentration of ethrel in leves of *Brassica juncea* (Mir *et al* 2008, Lone *et al* 2010). Das (2000) reviewed that cobalt plays important role in nitrogen fixation. Gad (2012) opined that amendment of cobalt at 12 ppm improved enzymes of nitrogen metabolism and improve yield. Kandil (2007) reported that cobalt addition increased the nodules formation of root and atmospheric nitrogen fixation by microorganisms which increase the nitrogen content in faba bean plants. Similar results were found by Abdel-Moez and Gad (2002).

ii) Phosphoenol Pyruvate (PEP) carboxylase

Phosphoenolpyruvate carboxylase [P_i: oxaloacetate carboxylase (phosphorylating)], first described by Bandurski and Greiner is now known to occur in all plants and plays a particularly important role in carbon fixation. The PEP carboxylase activity showed a decreasing trend from vegetative to post-flowering stage (Fig. 35).

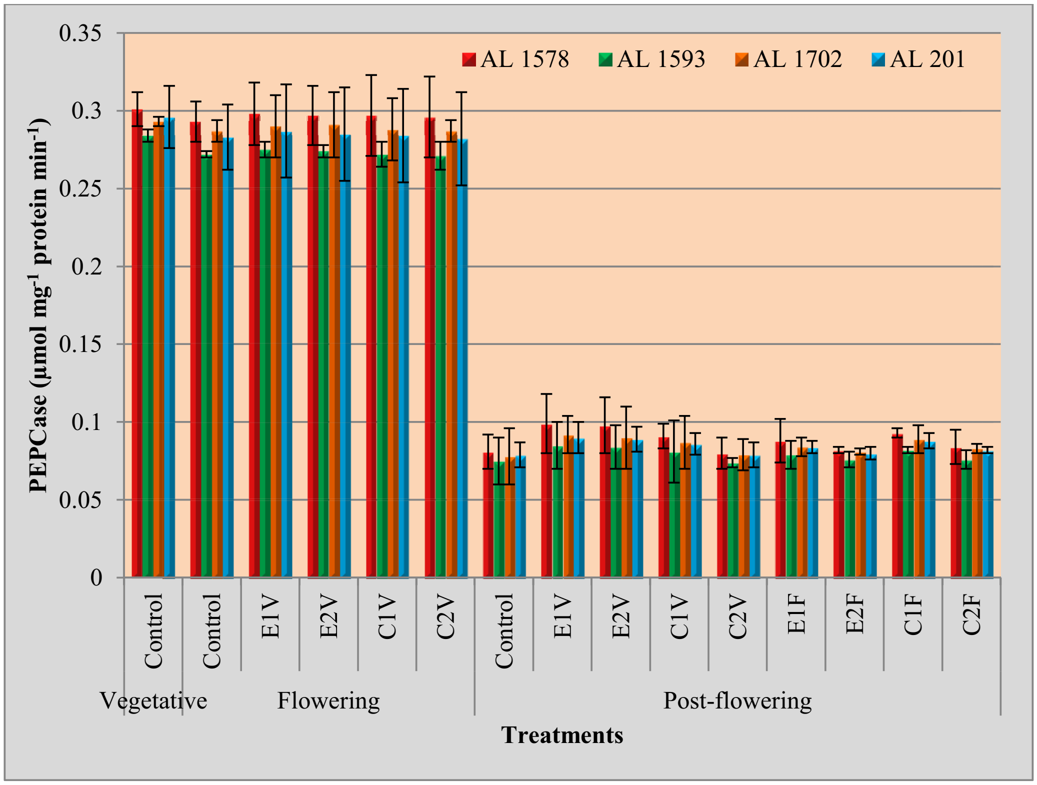


Fig. 35: Effect of foliar application of ethrel and cobalt chloride on PEPCase activity in leaves in pigeonpea genotypes

Among the treatments, the enzyme activity was maximum with E_1V treatment. At vegetative stage, activity of PEP carboxylase was maximum in AL 1578 (0.301 µmol mg^{-1} protein min^{-1}) followed by check genotype AL 201 (0.296 µmol mg^{-1} protein min^{-1}). The enzyme activity which indicates the enzyme potential was maximum in AL 1578, both at flowering and post flowering stage. At flowering stage, E_2V and C_1V differed non-significantly (on mean basis) and 0.285 µmol mg^{-1} protein min^{-1} activity was recorded with these treatments. At post-flowering stage E_1V and E_2V showed 16.67 and 15.38 % more activity than that of control.

In C_4 plants, the PEP carboxylse enzyme levels in leaves are about 20-fold higher on a Chlorophyll basis than in C_3 plants and the enzyme is localized in the cytoplasm of mesophyll cells. In addition to its role in C_4 and CAM plants, relatively higher levels of PEP2 carboxylase have been reported in several legumes. The enzyme has been suggested for reducing carbon losses by assimilating CO_2 released during dark respiration or photorespiration; hence playing a major role in dark fixation of CO_2. Legume seeds being rich in proteins require a large supply of amino acids, for the synthesis of which carbon skeletons are derived from tricarboxylic acid cycle. PEP carboxylase by playing an anapleurotic role in replenishing the intermediates of the above cycle might also be helping the synthesis of amino acids in developing seeds (Singal *et al* 1993). To achieve high yield, plants must establish optimal photosynthetic capacity and then maintain a high rate of photosynthesis during the grain filling period and nitrogen supply plays a major role in both processes by maximizing the biochemical components of the photosynthetic apparatus (Below 2002).

iii) Invertase activity in developing seeds

In developing seeds invertase activity declined from 10 DAF to 30 DAF in all treatments and genotypes. Different treatments enhanced the activity of invertase enzyme at different stages of development (Fig. 36).

At 10 DAF, in control plants, invertase activity was high in genotype AL 1702 (228.34 nmol g^{-1} min^{-1}) followed by AL 201 (222.80 nmol g^{-1} min^{-1}) and AL 1578 (220.57 nmol g^{-1} min^{-1}). Among treatments, E_2V (on mean basis) caused maximum increase in invertase activity (248.65 nmol g^{-1} min^{-1}) followed by E_1V (248.61 nmol g^{-1} min^{-1}) treatment. E_1V and E_2V differed non-significantly from each other. Treatments E_1V and E_2V treatments showed 12% increase in invertase activity over control plants. At 20 DAF, there was decline in invertase activity. Ethrel sprayed at vegetative stage (i.e. E_1V and E_2V) was most effective in maintaining high invertase activity than control and other treatments. Treatment E_1V and E_2V showed 9 and 8% increase over control respectively. At 30 DAF, similar trend was observed in activity of invertase, E_1V treatment showed highest invertase acivity in developing seeds than control and all other treatments.

Invertase enzyme is implicated as to play a major role in cleaving imported sucrose which in turn may regulate the rate of carbon import to the developing fruit (Robinson *et al* 1988). In our study, high activity of acid invertase in treated plants resulted in high biomass. It can be responsible for providing more reserves for the developing seed hence determining its strength. Afzal *et al* (2005) reported an increase in invertase activity in seeds of treated plants.

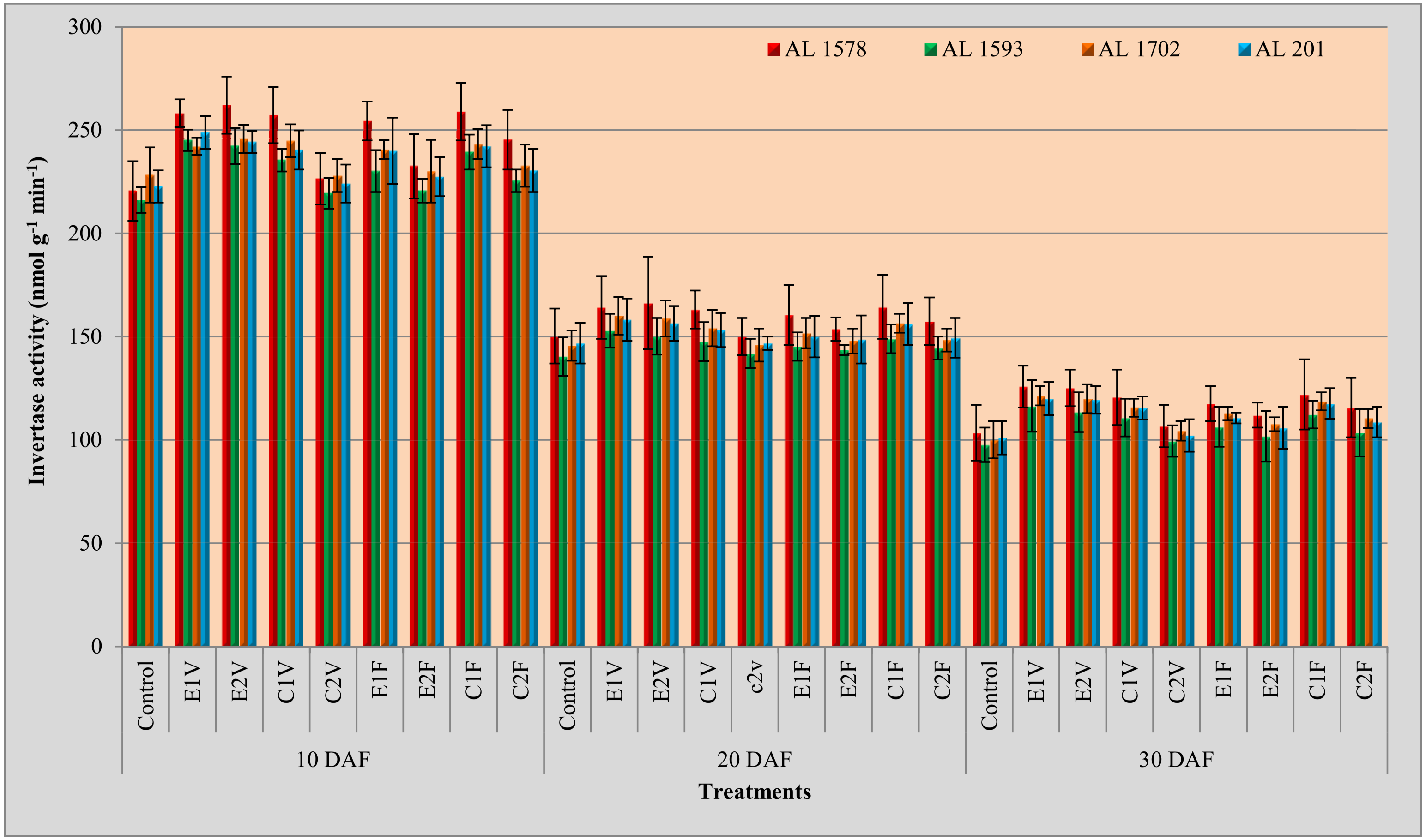


Fig. 36: Effect of ethrel and cobalt chloride on invertase activity in developing seeds of pigeonpea genotypes

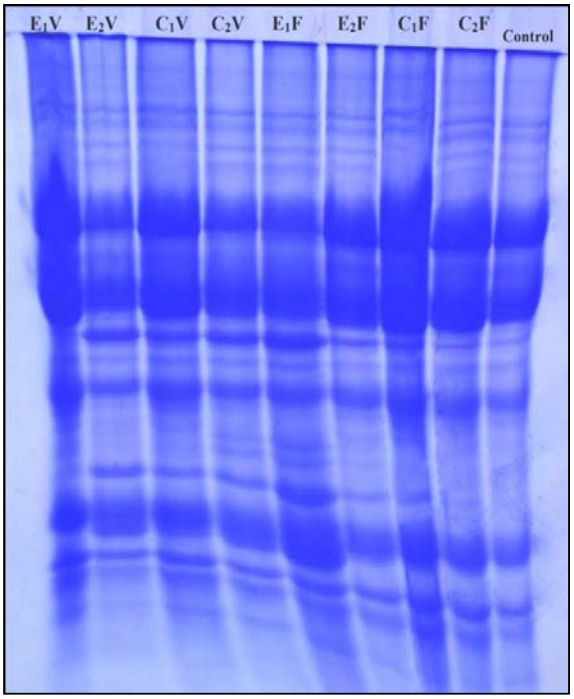
4.2.7 Protein profiling in mature seeds

For most seeds, except cereals, storage proteins are predominantly globulins (Krishna and Bhatia 1985). These mature seeds provide a stable and convenient system for biochemical analysis. The SDS-PAGE of total seed proteins was used to assess genetic variability of pigeonpea genotypes subjected to PGRs. The electrophoretic separation of these proteins and their densitometry analysis is illustrated in plate (1 and 2) and Fig. (37 and 38).

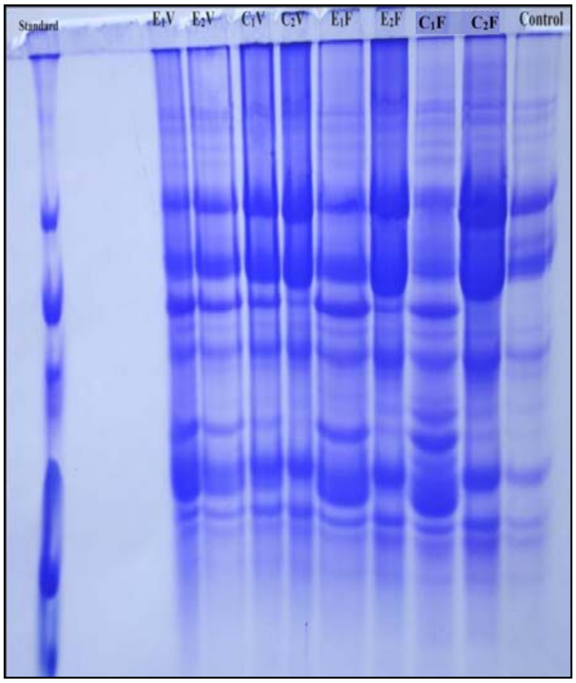
SDS-PAGE of seed proteins of pigeonpea genotypes resulted in scoring of maximum of 18 polypeptide bands. The results in general showed variation in number and intensity of protein bands. In all genotypes, the band number 1-4, 5-6, and 9 were monomorphically expressed in all treatments with light, dense and medium intensities. Electrophoretic analysis of protein patterns of pigeonpea showed that in genotype AL 1578, polypeptides with band number 1 (R_f 0.046), 2 (R_f 0.09), 3 (R_f 0.14), 4 (R_f 0.166), 5 (R_f 0.218), 6(R_f 0.337), 9 (R_f 0.505), 14 (R_f 0.738) and 15 (R_f 0.825) were most prominent in control (Fig 37). The effect of applied growth regulators resulted in induction of new bands i.e band number 11 (R_f 0.561) and 13 (R_f 0.654), however, the intensity of bands were more in E_1V, C_1V, C_2V, E_1F and C_1F treatments. There were similar protein profile for band number 7 (R_f 0.410), 10 (R_f 0.553), 12 (R_f 0.589) by all treatments. An over accumulation for protein band number 7 (R_f 0.410) and 14 (R_f 0.70) was observed in E_1V, E_2V, C_1V, C_2V, E_1F treatments. These results indicated that the effect of treatments lead to increase of some protein band intensities compared to other treatments. In genotype AL 1593, all treatments resulted in induction of band number 7 (R_f 0.442). Band number 11 (R_f 0.658) and 12 (R_f 0.745) were more intense in treatment C_1F and E_1V while in control and all other treatments its was of light intensity (Fig. 37). In genotype AL 1702, band number 8, 14, 17 and 18 were more intense in treatments as compared to control. New band of light intensity (band number 12) was appeared in all treatments except C_2V treatment. In genotype AL 201, band number 11 and 16 were new.

The overall result of total seed storage protein pattern obtained by SDS-PAGE were effective in studying genetic effect of each treatment in pigeonpea genotypes. The occurrence of newly synthesized bands and the absence of others in treated plants under different treatments apparently is indicative of either enhancement or repression of gene expression in these plants. This might alter the produced proteins in response to plant growth regulators either at the transcription or post transcription levels of gene expression. In the present study, applied growth regulators induced considerable variations in the protein patterns. These variations have been manifested as the novel expression of some polypeptides and the absence of others. A wide range of variations was found either in band density or intensities.

Several of new proteins synthesized in response to plant growth regulators have been reported as stress proteins in plants (Hoyos and Zhang 2000, Patharkar and Cushman, 2000).

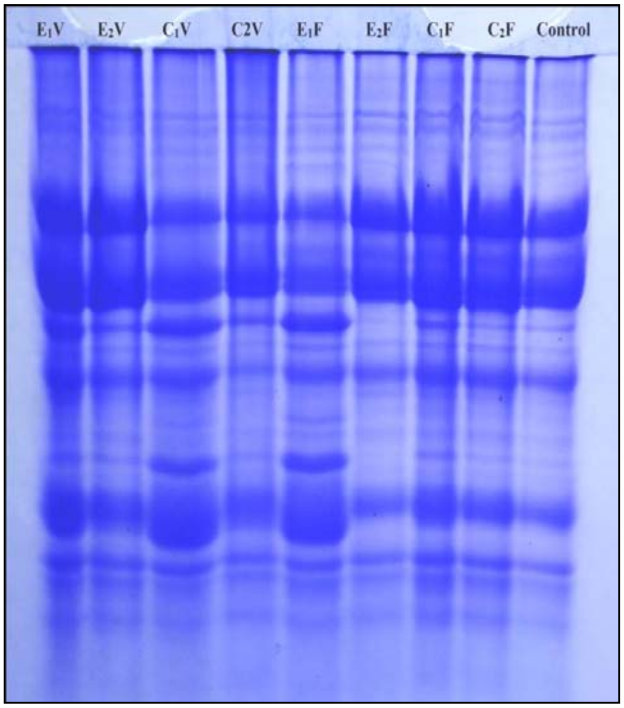


AL 1578

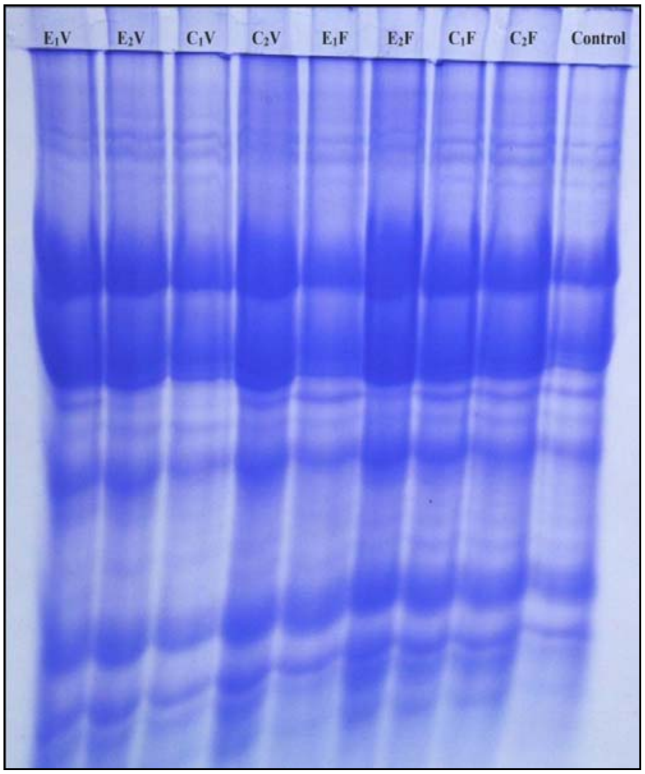


AL 1593

Plate1 Effect of ethrel and cobalt chloride on protein profiling in mature seeds of pigeonpea genotypes

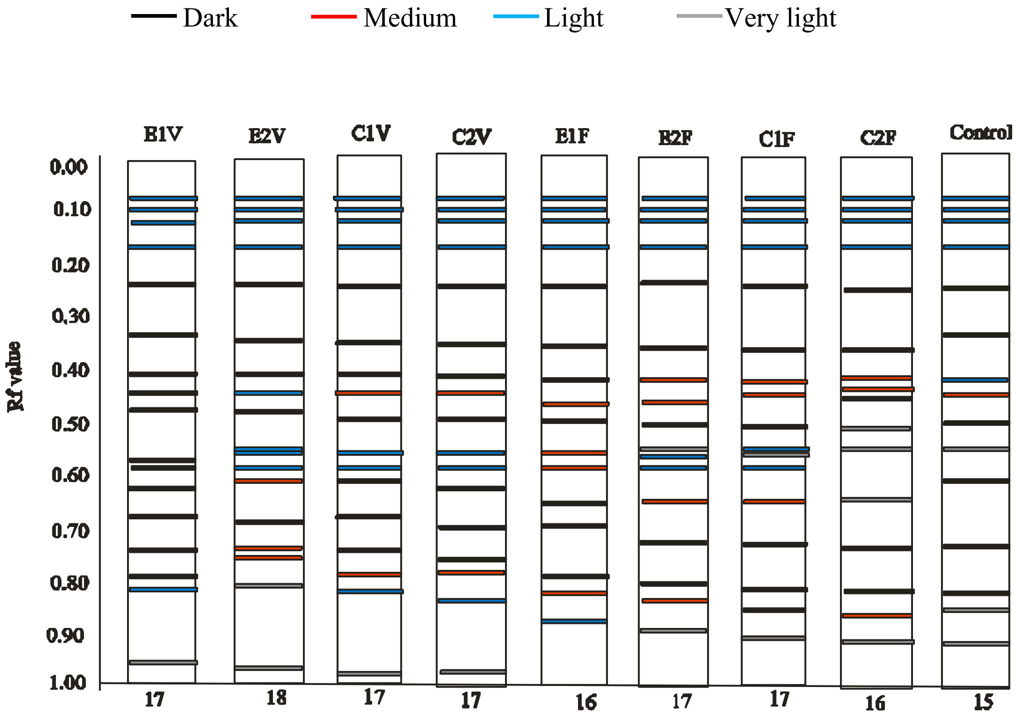


AL 1702

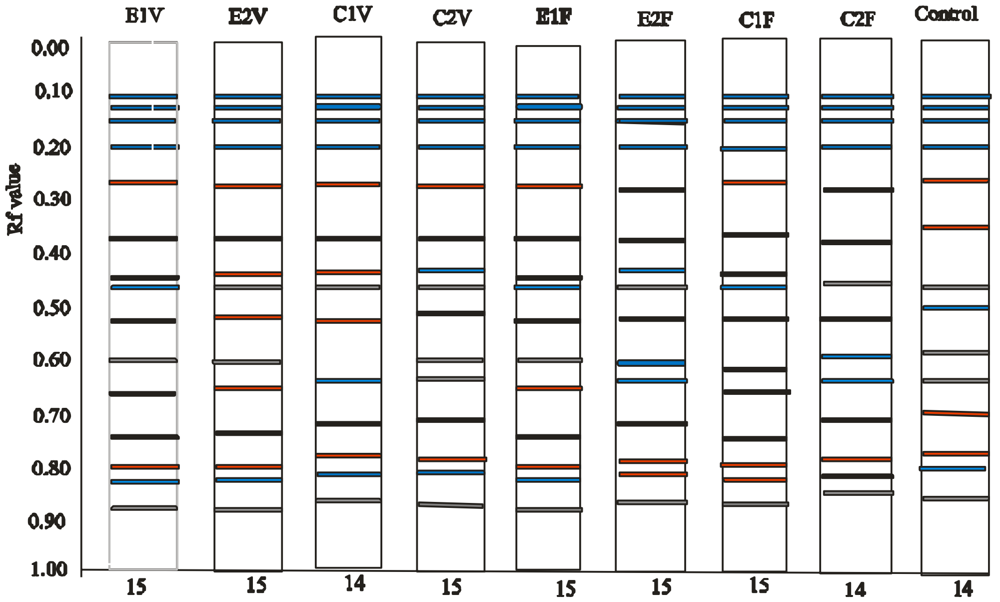


AL 201

Plate 2. Effect of ethrel and cobalt chloride on protein profiling in mature seeds of pigeonpea genotypes

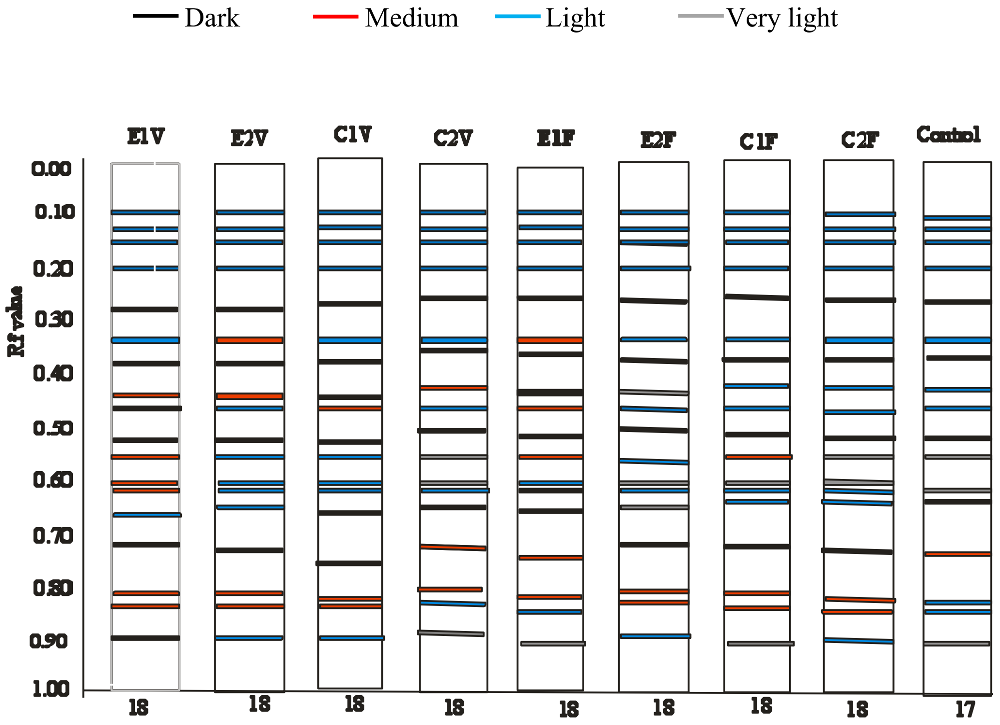


AL 1578

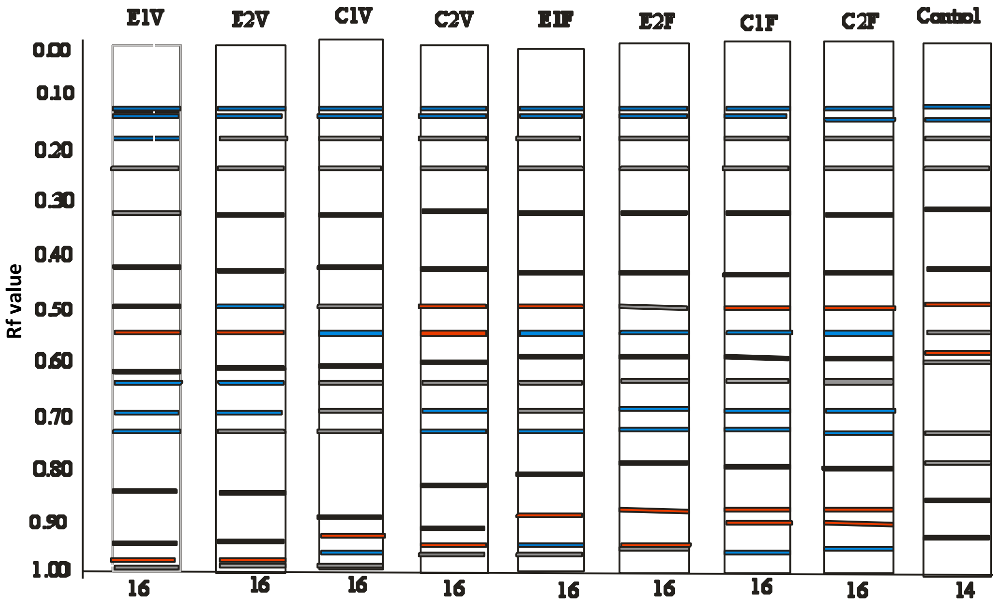


AL 1593

Fig. 37: Zymogram of total soluble seed protein of pigeonpea genotypes as influenced by plant growth regulators



AL 1702



AL 201

Fig. 38: Zymogram of total soluble seed protein of pigeonpea genotypes as influenced by plant growth regulators

These modifications in gene expression can be illustrated as plant response for plant growth regulators. In general, it can be concluded that the application of applied plant growth regulators affected the expression of different plant genes, where some of them were suppressed, while some novel proteins were induced. These effects were correlated with the type of plant growth regulators involved in the treatment, time of application and the dose, which reach to organ. Hossanien *et al* (2004) noted these changes as the effect of some herbicides on faba bean. Where some genes were promoted (up regulated) and gave intensive or normal band, while some others were suppressed (down regulated) and gave less intensive or absent band. Similar effect of PGRs on protein profiling of fababean was observed by Ibrahim *et al* (2007b).

From these results, it is concluded that there is a remarkable change in protein pattern in response to different plant growth regulators treatments. It appears that a number of polypeptides increased or decreased in intensity while others disappeared or new polypeptides were expressed. Most of these modifications appear to be a part of metabolic changes in response to developmental stages (Ibrahim *et al* 2005). Similar effect on protein pattern by biofertilizers application was observed by Selvakumar *et al* (2012).

4.2.8 Anatomical studies on the pedicel of pigeonpea

In order to study the influence of different growth regulators on translocatory tissue, especially phloem, the pedicels were sectioned and anatomical observations were undertaken in genotypes AL 1578 and AL 201 which were treated with ethrel and cobalt chloride at flowering stage (Table 21).

Table 21: Effect of ethrel and cobalt chloride on the structure of pedicel of the pigeonpea genotypes AL 1578 and AL 201

	Total area (in 10X view)				
	Diameter	**Cortex**	**Phloem fiber**	**Phloem**	**Xylem**
AL 1578					
Control	25.02	309.57	70.68	37.30	48.54
E_1F	25.83	276.29	99.27	75.12	43.06
E_2F	26.24	301.74	93.14	60.06	54.80
C_1F	26.74	318.60	119.47	73.47	48.44
C_2F	27.46	342.09	92.89	69.08	54.70
AL 201					
Control	25.12	327.56	66.13	45.21	35.87
E_1F	26.58	347.06	91.85	70.83	32.55
E_2F	25.87	342.49	81.70	52.06	38.38
C_1F	27.41	333.04	85.22	79.37	49.36
C_2F	29.41	393.07	87.32	62.33	69.76

The pedicels of the flowers contain small vascular bundles surrounded by a ring of fibres (Bisen and Sheldrake 1981, Begum *et al* 2007). The diameter of the pedicel and the area occupied by various tissues, viz. cortex, phloem fibres, phloem and xylem etc are depicted in Table 21

The diameter of the pedicel increased with all treatments. The area of the main translocatory tissue i.e. phloem increased significantly with all the treatments. Similar effect of plant growth regulators on translocatory tissue was observed by Kaur (1990).

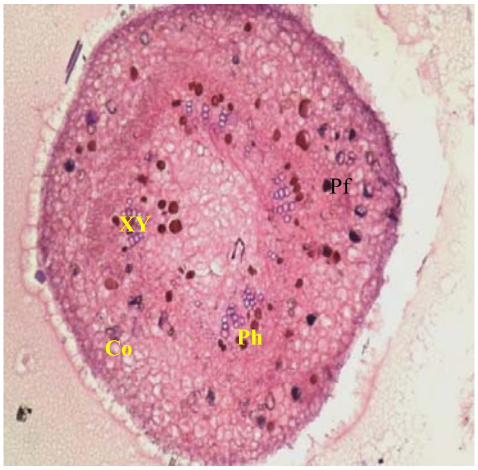
4.2.9 Correlation and path coefficient analysis of yield and yield related traits

Correlation matrix showed significant positive correlation between yield and related traits in pigeonpea genotypes (Table 22, 23; Fig. 39). Highest significant positive correlation was shown by number of branches/plant (r= 0.887**), CGR (r= 0.871), photosynthesis (r= 0.8945), number of flowers/plant (r= 0.6384), number of pods/plant (r= 0.937), pod set % (r= 0.8642) and pod weight/plant (r=0.910). It seems that by increasing pod weight due to more absorption of photoassimilates, the most portion of assimilates remobilizes to seeds, so the grain weight get increased. The pod set percentage was highly significantly correlated with number of branches/plant, CGR, photosynthesis and number of pods/plant and hence simultaneous selection for these traits might bring improvement in yield.

Seed yield depends upon several traits which are mutually related. Therefore, a change in any one of the components would ultimately disturb the complex relationship. Hence, these traits have to be analyzed for their action viz. direct effect of component trait on seed yield and the indirect effects through other component traits on seed yield. Path analysis was used to partition the correlation matrix into causal and spurious effects and then the causal effect was partitioned into direct and indirect effects. The path coefficient analysis revealed that highest positive direct effect on seed yield was exhibited by number of branches/plant (P_1= 0.2368) followed by pod set % (P_6= 0.209), number of pods/plant (P_5= 0.1866), pod weight/plant (P_7= 0.1433), photosynthesis (P_3= 0.1249) respectively. Hence, these traits could be relied upon , for the selection of genotypes to improve yield potential of pigeonpea. The direct effect of CGR (P_2 =0.0519) was very low, while its high correlation value (r=0.871) in correlation matrix was due to its indirect contribution via other traits studied.

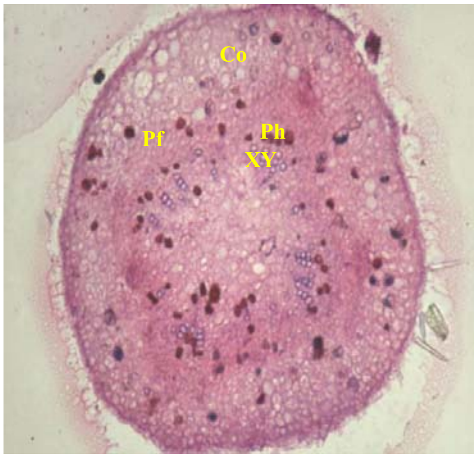
Further, in the present investigation, it was evident that 90% of yield constituting traits were utilized in this analysis as the residual value which determine how best the causal variables account the variability in seed yield or which represent the effect of other factors or variables not considered in the path analysis was very low (0.080).

Control

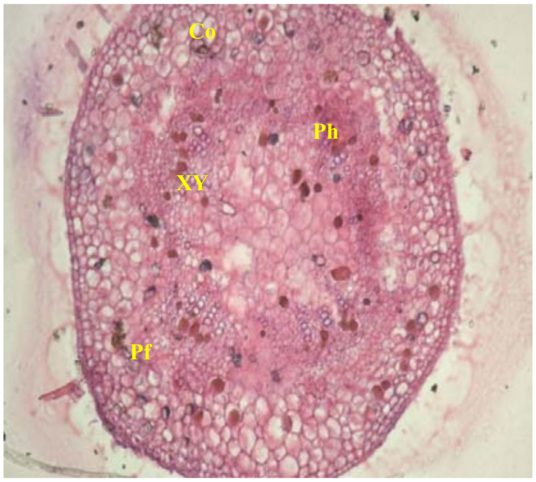


E_1F

E_2F

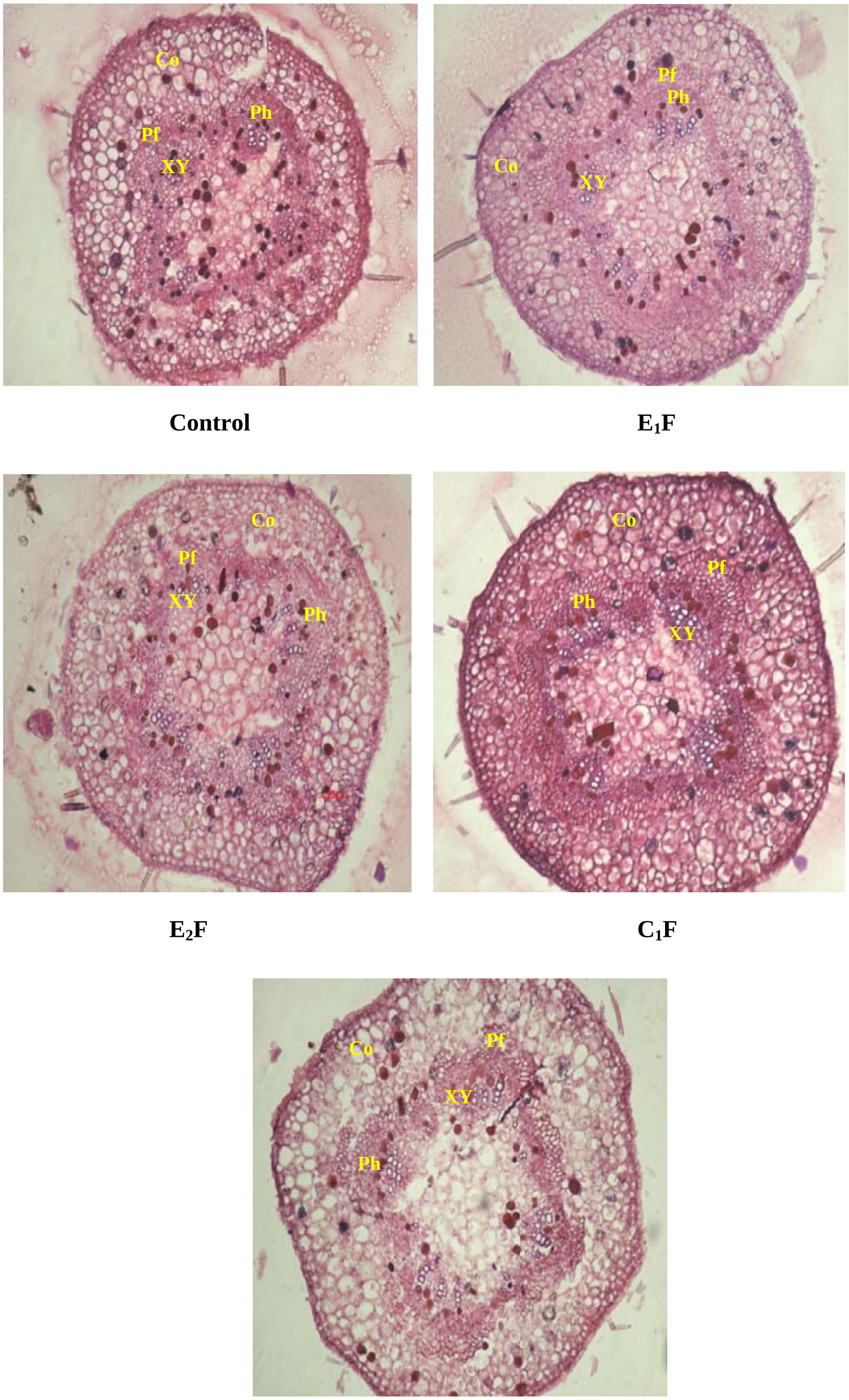


C_1F



C_2F

Plate 3: Transverse sections of pedicel of flower of the pigeonpea genotype AL 1578 (XY= Xylem, Ph= Phloem, Pf= Phloem fibers, Co= Cortex)



Control

E_1F

E_2F

C_1F

C_2F

Plate 4: Transverse sections of pedicel of flower of the pigeonpea genotype AL 201 (XY= Xylem, Ph= Phloem, Pf= Phloem fibers, Co= Cortex)

Table 22: Correlation matrix of yield and yield related traits in pigeonpea genotypes

S. No.	Charcters	Number of branches	CGR	Photosynthesis	Number of flowers	Number of pods	Pod set %	Pod weight/plant
1	Number of branches	1						
2	CGR	0.7956**						
3	Photosynthesis	0.8548**	0.8581**					
4	Number of flowers	0.4724**	0.5879**	0.4522*				
5	Number of pods	0.8438**	0.8623**	0.8574**	0.7655**			
6	Pod set %	0.7941**	0.7852**	0.8936**	0.3797*	0.8398**		
7	Pod weight/plant	0.8460**	0.8890**	0.8454**	0.6702**	0.9103**	0.7821**	
8	Yield	0.8868**	0.8712**	0.8946**	0.6382**	0.9370**	0.8641**	0.9100**

**Significant at 1%, *Significant at 5%

Table 23: Path analysis of yield and yield related traits in pigeonpea genotypes

S. No.	Characters	Number of branches	CGR	Photosynthesis	Number of flowers	Number of pods	Pod set %	Pod weight/plant	Correlation
1	**Number of branches**	**0.2368**	0.0413	0.1068	0.0573	0.1574	0.166	0.1212	0.8868
2	**CGR**	0.1884	**0.0519**	0.1072	0.0713	0.1609	0.1641	0.1274	0.8712
3	**Photosynthesis**	0.2024	0.0445	**0.1249**	0.0549	0.16	0.1868	0.1211	0.8946
4	**Number of flowers**	0.1118	0.0305	0.0565	**0.1213**	0.1428	0.0794	0.096	0.6383
5	**Number of pods**	0.1998	0.0447	0.1071	0.0929	**0.1866**	0.1755	0.1304	0.937
6	**Pod set %**	0.188	0.0407	0.1116	0.0461	0.1567	**0.209**	0.112	0.8641
7	**Pod weight/plant**	0.2003	0.0461	0.1056	0.0813	0.1699	0.1635	**0.1433**	0.9100

Bold (direct effect), Residual (0.080)

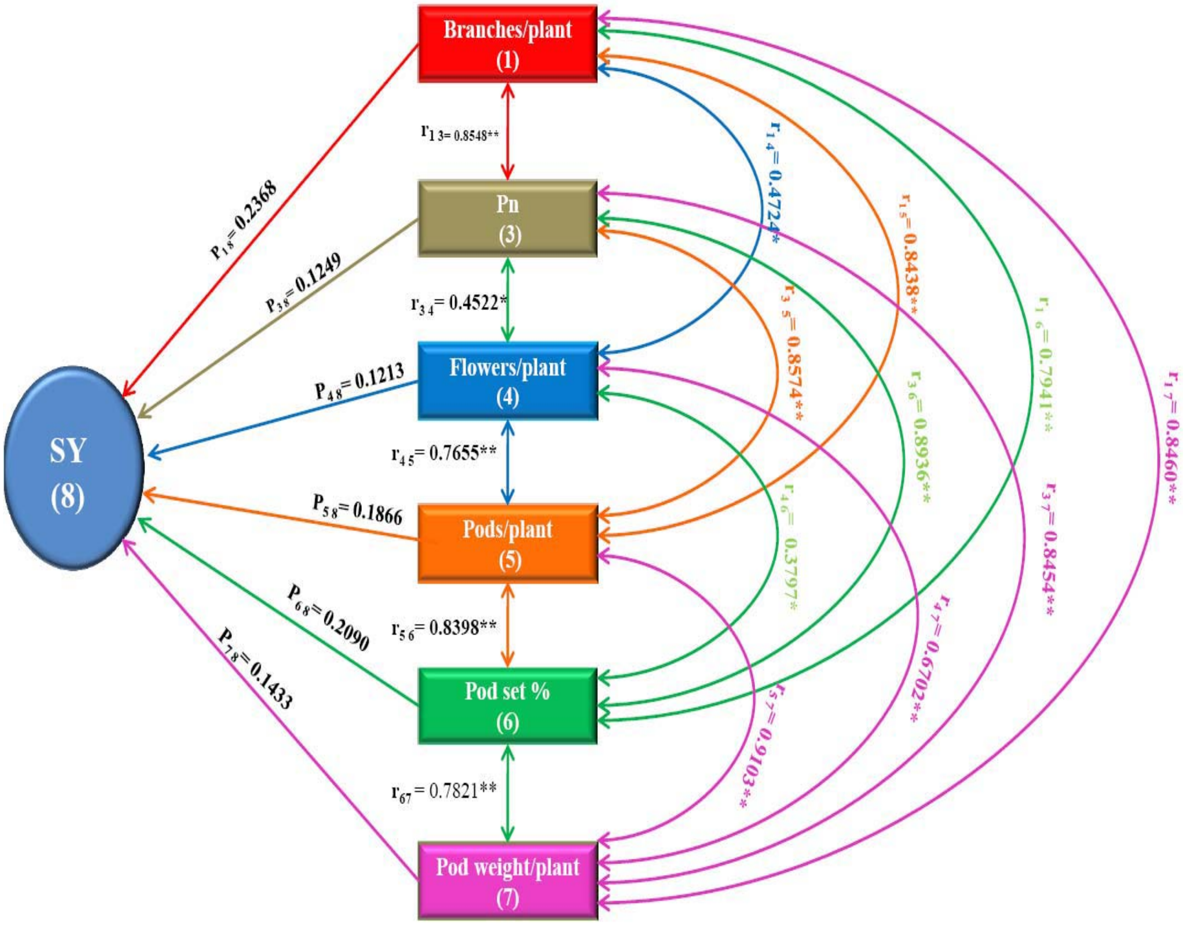


Fig. 39: Path diagram showing the relationship between grain yield and different traits. (Where, single headed arrows indicate direct effects, double headed arrows indicate simple correlation;Significant at 1%)**

Our results are in agreement with Padi (2003). Sodavadiya *et al* (2009) proposed that the information on association of different components with yield and among themselves is of considerable importance in selection programme. A study on correlation alone is not enough to give exact picture of relative importance of direct and indirect influence of each of the component traits on seed yield. Similar results were obtained by Patel *et al* (2009).

4.2.10 Correlation and path coefficient analysis of yield and biochemical traits

Correlation matrix revealed significant positive correlation of yield with various biochemical traits except PEPCase activity in leaves, where the correlation was positive but it was non-significant (Table 24, 25 and Fig. 40). The seed yield per plant was significantly and positively correlated with nitrate reductase activity (r=0.770), invertase activity in seeds (r=0.9372), TSS in leaves (r=0.8843), amino acids in leaves (r=0.874), proteins in leaves (r=0.8676), sugars in seeds (r=0.8479) and starch in seeds (r=0.6431). Such positive interrelationship between yield and biochemical traits were also reported by Kaur (2012). Thus, providing evidence that these attributes were more influencing the seed yield in pigeonpea and they can serve as important traits for improvement in seed yield.

The path coefficient analysis provides a more realistic picture of the interrelationship, as it considers direct as well as indirect effects of the variables by partitioning the correlation coefficient. Path coefficient analysis in the present study revealed that nitrate reductase, PEPCase, invertase in seeds, starch content in leaves, amino acids in leaves, proteins in leaves and starch in seeds exhibited positive direct effects. Thus, these characters turned out to be the major componenets of seed yield. Other traits showed significant correlation with yield due to indirect contribution through other traits. Total soluble sugars showed significant negative direct effect. However, in correlation matrix, it showed positive correlation due to indirect contribution through other traits.

The residual effect on seed yield was very low indicating that most of the biochemical contributing characters were included in the path analysis. Sodavadiya *et al* (2009) also reported low residual effect.

Table 24: Correlation of yield and biochemical traits in pigeonpea genotypes

S. No.	Characters	Nitrate reductase in leaves	PEPCase activity in leaves	Invertase activity in seeds	TSS in leaves	Starch in leaves	Amino acids in leaves	Proteins in leaves	Sugars in seeeds	Starch in seeds
1	**Nitrate reductase activity in leaves**	1								
2	**PEPCase activity in leaves**	-0.257								
3	**Invertase activity in seeds**	0.8020**	0.1282							
4	**TSS in leaves**	0.6955**	0.2704*	0.8937**						
5	**Starch in leaves**	0.6598**	0.3159*	0.8691**	0.9610**					
6	**Amino acids in leaves**	0.7207**	0.1066	0.8377**	0.8621**	0.8874**				
7	**Proteins in leaves**	0.6801**	0.2011	0.8943**	0.8946**	0.8610**	0.7443**			
8	**TSS in seeds**	0.6312**	0.2047	0.6060**	0.9032**	0.9369**	0.9016**	0.7685**		
9	**Starch in seeds**	0.4582*	0.1768	0.6110**	0.6150**	0.6669**	0.6659**	0.4535*	0.7598**	
10	**Yield**	0.7700**	0.2170	0.9372**	0.8726**	0.8843**	0.8740**	0.8676**	0.8479**	0.6431**

**Significant at 1%, *Significant at 5%

Table 25: Path analysis of yield and biochemical traits in pigeonpea genotypes

S. No.	Characters	Nitrate reductase in leaves	PEPCase activityin leaves	Invertase activity in seeds	TSS in leaves	Starch In leaves	Amino acids in leaves	Proteins in leaves	Sugars in seeeds	Starch in seeds	Correlation
1	**Nitrate reductase**	**0.0569**	-0.0024	0.4666	-0.2320	0.1962	0.2564	0.1436	-0.1593	0.0509	**0.7769**
2	**PEPCase activity in leaves**	-0.0015	**0.0917**	0.0746	-0.0902	0.0939	0.0379	0.0425	-0.0517	0.0196	**0.2168**
3	**Invertase activity in seeds**	0.0456	0.0118	**0.5819**	-0.2981	0.2585	0.2980	0.1889	-0.2172	0.0679	**0.9373**
4	**TSS in leaves**	0.0395	0.0248	0.5200	**-0.3335**	0.2858	0.3067	0.1889	-0.2279	0.0684	**0.8727**
5	**Starch in leaves**	0.0375	0.0290	0.5057	-0.3205	**0.2974**	0.3157	0.1818	-0.2364	0.0741	**0.8843**
6	**Amino acids in leaves**	0.0410	0.0098	0.4874	-0.2875	0.2639	**0.3558**	0.1572	-0.2275	0.0740	**0.8741**
7	**Proteins in leaves**	0.0387	0.0184	0.5204	-0.2984	0.2560	0.2648	**0.2112**	-0.1939	0.0504	**0.8676**
8	**TSS in seeds**	0.0359	0.0188	0.5008	-0.3012	0.2786	0.3207	0.1623	**-0.2524**	0.0845	**0.8480**
9	**Starch in seeds**	0.0260	0.0162	0.3555	-0.2051	0.1983	0.2369	0.0958	-0.1917	**0.1112**	**0.6431**

Bold (direct effect), Residual (0.095)

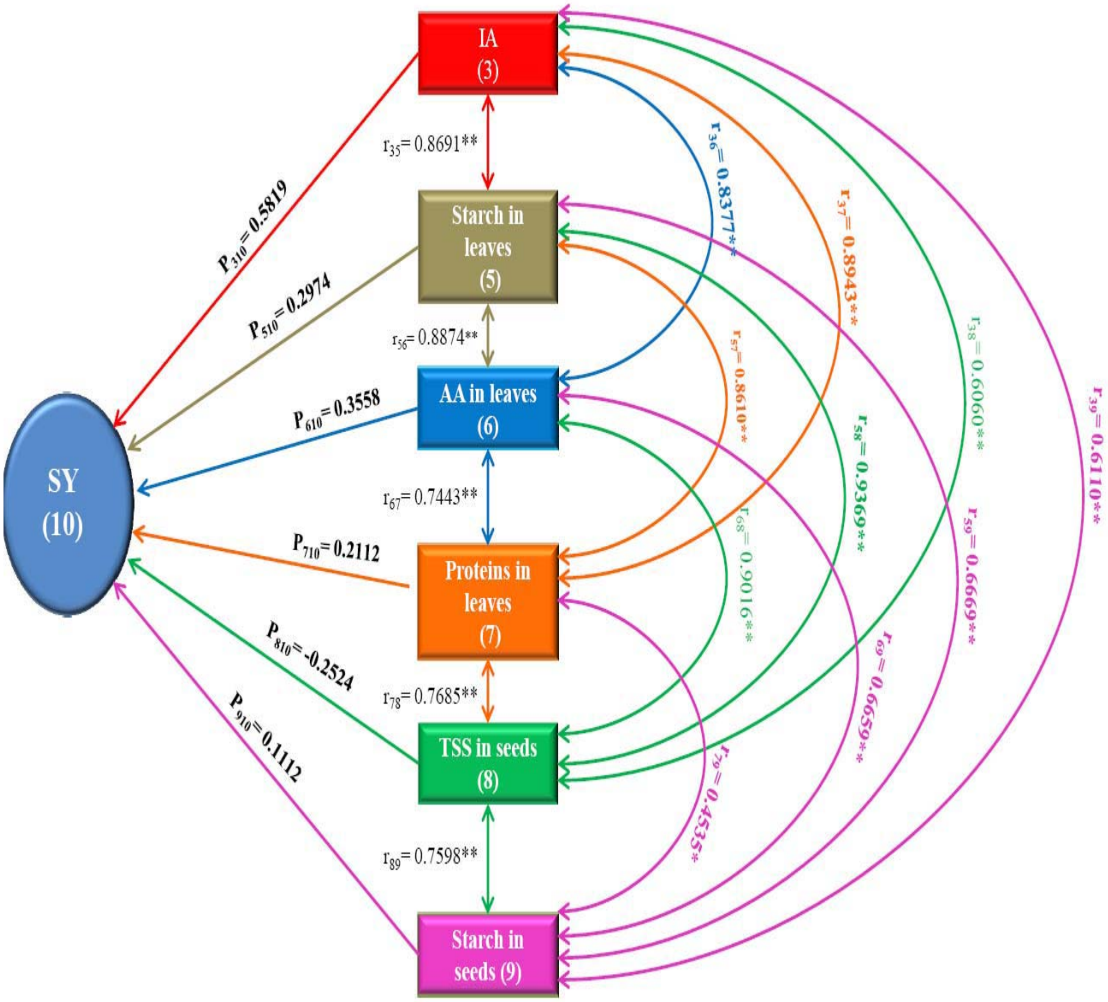


Fig. 40: Path diagram showing the relationship between grain yield and different traits. (Where, single headed arrows indicate direct effects, double headed arrows indicate simple correlation;Significant at 1%)**

Thus, it is inferred that ethrel and cobalt chloride when used at appropriate time and in appropriate concentration can improve the yield potential in pigeonpea. A significant improvement in morpho-physiological traits, growth parameters, biochemical constituents and yield and yield components was observed due to application of these PGRs.

The increase in yield could be a reflection of the effect of bioregulators in inhibition of flower abscission, improvement in morpho-physiological characters and enhanced dry matter production. The enhanced dry matter production could be related to:

- Increase in carbohydrate metabolism, improved source-sink relationship and
- Protein accumulation which leads to better flower and pod retention and hence yield.
- Also, of the four genotypes used for yield improvement through PGRs application AL 1578 performed best followed by AL 1702, AL 201 and AL 1593. Among the treatments, E_1V followed by E_2V and C_1F treatment were most effective in retaining maximum number of flowers, thereby resulting in effective pod formation and ultimately improving yield in all the genotypes.

CHAPTER V

SUMMARY

Pigeon pea (*Cajanus cajan* L.) is a multipurpose legume crop and is well adapted even in marginal lands. In India, it is second in area and 91% of world's pigeonpea is produced in India. Pigeonpea has a wide range of products, including the dried seeds, pods and immature seeds used as green vegetables, leaves and stems used for fodder and dry stem as fuel. Pigeonpea is known to prevent and cure many diseases. Dhal has 25% protein with a good balance of all amino acids except methionine and cysteine (Fu *et al* 2007).

The crop though accumulates maximum biomass, yet proportionally low final yield is obtained due to higher partitioning of dry matter towards vegetative growth. Excessive vegetative growth, indeterminate growth habit, flower fall and poor source-sink relationship seems to be major constraint in high yield of pigeonpea (Chudasama and Thaker 2007). Its potential for high yield and wide adaptability can contribute to briging the pulse shortage, if higher partitioning of dry matter is accomplished towards the storage sinks (Sharma *et al* 2009). The improvement of source-sink relationship can be achieved by PGRs. Ethylene and $CoCl_2$ are known to improve source-sink relationship when used in appropriate concentration and specific time (Coll *et al* 2001, Basu *et al* 2006).

Thus, the present investigation was conducted to study the various physiological parameters in 25 pigeonpea genotypes differing in maturity duration (Early, Mid, Late and check) for differential flowering behavior and to study the effects of different concentrations of ethrel and cobalt chloride sprayed at vegetative and flowering stage on manipulation of source-sink relationship and improvement of yield in four mid-duration genotypes (AL 1578, AL 1593, AL 1702 and AL 201) . The salient findings are:

Experiment I

- Plant height and number of branches/plant were maximum in mid and late duration genotypes while early duration genotypes had minimum plant height.
- Growth efficiency parameters such as RGR showed decline at stage II while CGR showed increase. The maximum CGR was noticed in mid duration genotypes.
- Leaf characteristics, viz. leaf area, leaf area index (LAI), specific leaf area (SLA) and specific leaf weight (SLW) varied significantly between different groups of genotypes. All these characters were maximum in mid duration genotypes while early genotypes had minimum.
- Photosynthetic rate and stomatal conductance were found to be higher in mid duration genotypes as compared to all other genotypes and was minimum in early duration genotypes.

- Phenological traits, such as days to 50% flowering were lesser in early duration group as compared to mid and late duration group of genotypes.
- Number of flowers/plant showed less difference among various groups. However, significant differences were observed in number of pods/plant. Mid duration genotypes had highest number of pods/plant and minimum was found in early duration genotypes.
- Pod set percentage was maximum in mid duration group followed by check and late duration group and was found to be minimum in early duration group.
- Pod weight/plant, seed weight/plant and partitioning coefficient were higher in mid duration group and lowest in early duration group.
- Harvest index and seed yield (kg/ha) was maximum in mid duration genotypes and least in early duration genotypes.
- Among early duration group AL 1757 and AL 1758 , among mid duration group AL 1744, AL 1578 , AL 1702 , among late duration group AL 1721, AL 1792 and among checks P992, AL 201 exhibited better growth in terms of leaf area, LAI, number of pods/plant, seed weight/plant and harvest index.
- Correlation analysis showed significant positive relationships between plant height, crop growth rate, leaf area, number of pods/plant, pod set percentage, seed weight/plant, 100-seed weight and harvest index. Further, path analysis revealed leaf area, specific leaf weight and number of pods/plant had significant positive direct influence on yield.

Experiment II

- Reduction in plant height was recorded in almost all treatments as compared to that of control.
- Number of branches/plant were higher with low concentration of ethrel sprayed at vegetative stage as compared with other treatments.
- Growth efficiency parameters such as crop growth rate and relative growth rate were estimated within different phases of growth and these parameters were improved in treated plants as compared to control.
- Various leaf characteristics viz. leaf area, leaf area index (LAI), specific leaf weight (SLW) and specific leaf area (SLA) enhanced with foliar application of PGRs at specific stage thereby strengthening the role of PGRs at particular developmental stage.
- The photosynthetic rate and stomatal conductance were maximum in treated plants as compared to control.

- Number of days to 50% flowering were less in ethrel treated plants at pre-flowering stage as compared to other treatments and control.
- Partitioning of dry matter towards reproductive parts (sink) was more in treated plants as compared to control. The PGRs stimulated the flow of assimilates from the vegetative to reproductive sinks.
- The increased pod set percentage showed influence on harvest index and yield of crop. The shift in balance of dry matter towards pods favours increased pod setting, pod and seed weight/plant and hence yield.
- Biochemical parameters viz. total soluble sugars, starch, amino acids, proteins in developing leaves and seeds were more in treated plants as compared to control. Increased protein turnover from exporting leaves to developing seeds was more in ethrel treated plants which were sprayed at vegetative stage. In genotype AL 1578, these biochemical reserves were more as compared to other genotypes and were minimum in AL 1593.
- Higher leaf nitrate reductase and PEPCase activity was found in treated plants as compared to control. High activity of PEPCase following foliar spray seems to provide additional pyruvate for increased metabolic activity.
- Carbon metabolizing enzyme invertase also showed enhanced activity with foliar application of different PGRs.
- Protein profiling in mature seeds showed maximum number of bands in treated plants as compared to control. The intensity of bands was more in treated plants as compared to control.
- Anatomical study of pedicel of flower revealed an increase in area of conducting tissues through PGRs application.
- Path analysis was performed to partition the correlation matrix into direct and indirect effect. Path coefficient analysis showed that number of branches/plant, pod set percentage, invertase activity in developing seeds, starch content in leaves, amino acids in leaves and total soluble proteins in leaves showed maximum direct contribution to yield. Therefore, these traits should be used as target traits to improve seed yield.

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