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Influence of ethylene and cobalt chloride on photosynthetic parameters and pedicel anatomy of pigeonpea (*Cajanus cajan* L.) genotypes

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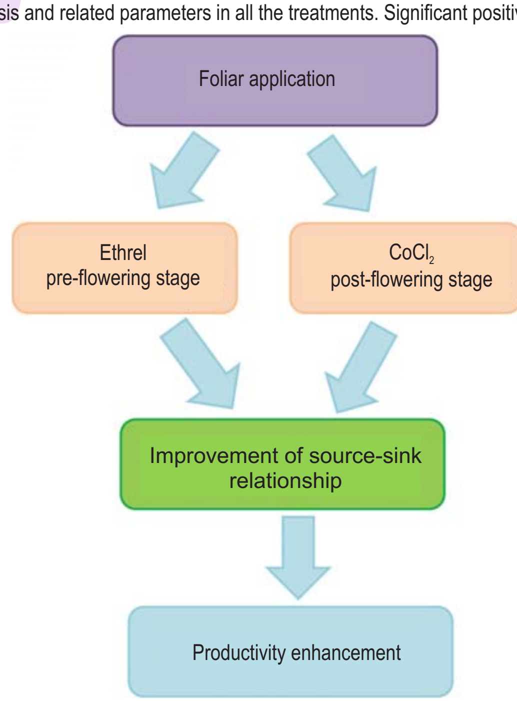
Abstract

Aim: A study was conducted with an aim to observe the effect of foliar application of ethylene (100 and 200μgml^{-1}) and $CoCl_2$ (10 and 15μgml^{-1}) at pre-flowering and post-flowering stages on different photosynthetic parameters *viz*., photosynthetic rate, internal CO_2 concentration, stomatal conductance, chlorophyll kinetics (Fv/Fm), total chlorophyll content and anatomy of pedicel of flower.

Methodology: Crop was foliarly sprayed with ethylene (100 and 200μgml^{-1}) and $CoCl_2$ (10 and 15μgml^{-1}) at pre-flowering and post-flowering stages. All parameters were recorded at vegetative, flowering and post flowering stages. Microtomy was done from pedicel of flower to observe the effect of treatments on conducting tissue.

Results: Different treatments significantly increased the photosynthesis related parameters. Lower concentration of ethrel sprayed at pre- flowering stage and cobalt chloride sprayed at post-flowering stage was most effective in increasing these parameters, and hence yield of crop. Maximum rate of photosynthesis and chlorophyll content was observed at flowering stage. Among the genotypes, AL 1578 recorded highest rate of photosynthesis and related parameters in all the treatments. Significant positive correlation of photosynthetic rate was observed with chlorophyll content and specific leaf weight. From the relationship between SLW and chlorophyll content with photosynthesis, it was observed 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. The area of conducting tissues viz. xylem and phloem in pedicel of flower was found to be maximum in treated plants which led to increased translocation of photosynthates and hence, seed yield.

Interpretation: Ethrel (100 μgml^{-1}) sprayed at pre- flowering stage and cobalt chloride (10 μgml^{-1}) sprayed at post-flowering stage was most effective in increasing photosynthesis and related parameters and hence, yield of crop.



Introduction

Pigeonpea (*Cajanus cajan* L.) is an important legume of tropics and sub-tropics. It has a wide range of products, including dried seeds, pods and immature seeds used as green vegetables, leaves and stems used for fodder and dry stems as fuel. Besides its nutrition value, pigeonpea also possesses various medicinal properties due to presence of a number of polyphenols and flavonoids. It has 25 % protein with good balance of all the amino acids except for methionine and cysteine (Zu *et al.,* 2006). Pigeonpea yield is low due to excessive vegetative growth, indeterminate growth habit, lack of moisture stress tolerance, poor source sink relationship, poor harvest index and poor biomass production (Chudasama and Thaker, 2007). However, the yield of crop can be increased by altering these parameters. Plant growth substances play a pivotal role in growth and differentiation of plants. When applied exogenously, these compounds induce several physiological and biochemical alterations that generally lead to morphological modifications with consequent effect on crop yield. In recent years, the promotory effect of growth retardants has been studied (Setia *et al.,* 1995).

Ethylene, a gaseous hormone has long been recognized as a growth inhibitor, but few studies have reported 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 radial expansion and horizontal orientation (Coll *et al.,* 2001). Ethylene released from ethrel (2-Chloroethyl phosphonic acid) is possibly 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 as it is used by micro-organisms for 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).

Therefore, effect of foliar application of ethrel and cobalt chloride sprayed at different growth stages on photosynthetic parameters and pedicel anatomy was investigated.

Materials and Methods

Seeds of pigeonpea genotypes (AL 1578, AL 1593, AL 1702 and AL 201) were procured from the Department Of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. These were foliarly sprayed twice with ethrel (100 and 200 µg ml^{-1}) and $CoCl_2$ (10 and 15 µg ml^{-1}) at vegetative and flowering stages and all the spray treatments were repeated after two days. Foliar treatments are summarized as follows:

E_1V: 100µgml^{-1} ethrel and E_2V-200µgml^{-1} ethrel at pre-lowering stage; E_1F: 100µgml^{-1} ethrel and E_2F-200µgml^{-1} ethrel at flowering stage; C_1V: 10 µg ml^{-1} $CoCl_2$ and C_2V: 15 µg ml^{-1} $CoCl_2$ at pre-flowering stage; C_1F: 10 µg ml^{-1} $CoCl_2$ and C_2F- 15 µg ml^{-1} $CoCl_2$ at flowering stage.

Different photosynthetic parameters *viz*., photosynthetic rate, internal CO_2 concentration, stomatal conductance, chlorophyll kinetics (Fv/Fm), total chlorophyll content were measured at vegetative, flowering and post-flowering stages. Photosynthetic rate, internal CO_2 concentration and stomatal conductance was measured from fully expanded leaf (third leaf from top) with a portable photosynthesis system (LICOR) assembled with an Infra Red Gas Analyzer (IRGA). Chlorophyll kinetics (Fv/Fm) was measured using chlorophyll florescence meter. For comparing pedicel anatomy of control and treated plants, flowers were fixed in formalin acetic acid (FAA) solution at flowering stage. The fixed material was processed for microtomy, sectioned at 10 µm thickness (Sass, 1958). Photomicrographs were taken on bright field research microscope fitted with digital camera and computer imaging system (Leica Bright Field Research Microcope, Germany). The data on various parameters were subjected to statistical analysis. Critical difference values were calculated through analysis of variance.

Results and Discussion

The production of biomass and grain yield of pulse crops can largely be accounted for 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 photosynthesis rate.

At vegetative stage, genotype AL 201, recorded maximum photosynthetic rate (8.63 µmol CO_2 m^{-2} s^{-1}) than all the other genotypes (Fig.1). At flowering stage, all the treatments recorded higher rate of photosynthesis as compared to control. On average of all the 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 the treatments applied except in treatment C_2V where AL 1593 showed 10.45 % higher net photosynthetic rate as compared to AL1578.

At pod development stage, net rate of photosynthesis decreased in all the 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

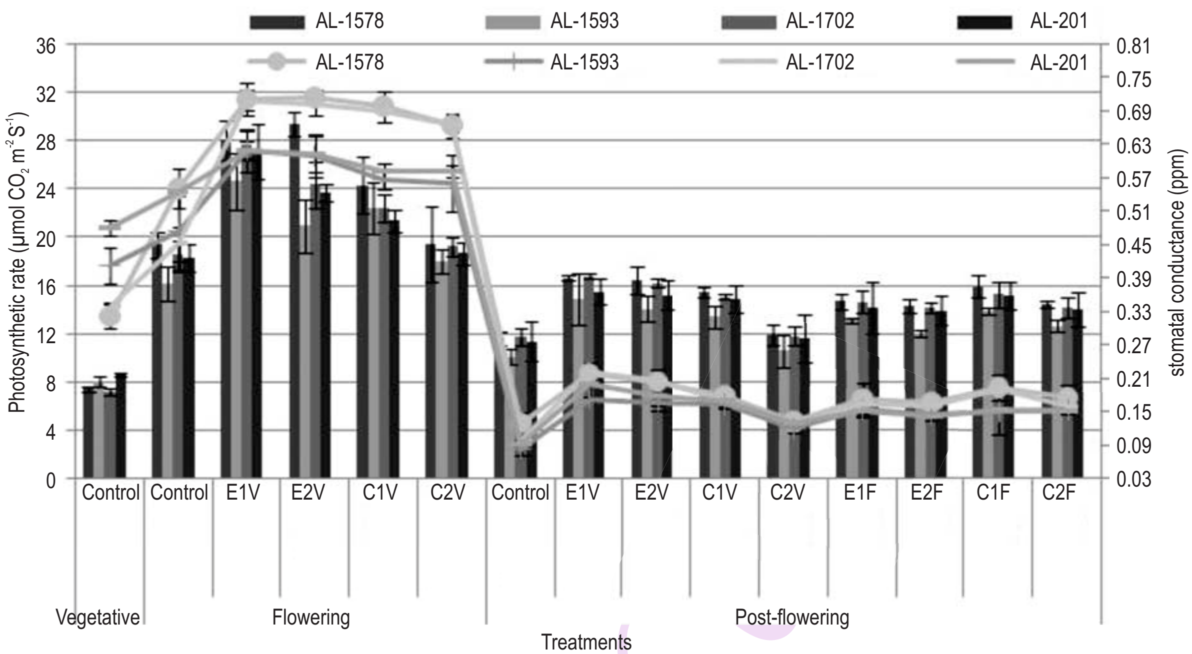


Fig. 1 : Effect of ethrel and cobalt chloride on photosynthetic rate and stomatal conductance in pigeonpea genotypes

Table 1 : 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				

photosynthetic rate was higher 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 minimum in AL 1593 (12.73 µmol $CO_2 m^{-2} s^{-1}$).

Mir *et al.* (2010) reported that 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 in *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 seed yield. Jaleel *et al*., (2009) reported that increased chlorophyll content at lower cobalt level was obvious due to better growth and hence, resulted in high photosynthetic activity.

Internal CO_2 concentration (C_i) values differed in all the genotypes and was maximum in genotype AL 1578 while, among the treatments none of the treatment showed significant effect over control (Table 1). At flowering stage, E_1V treatment showed highest C_i (307.02 ppm) followed by control (305.74 ppm) and lowest was recorded in C_2V treatment (285.89 ppm). At post flowering stage, C_i was less as compared to flowering stage. Control showed highest C_i (289.95) than all the treatments applied (289.95ppm). Various treatments exhibited significant differences in stomatal conductance. It showed a similar trend as that of net photosynthetic rate (Fig. 1). 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 AL1702. These results are in agreement with the study of Hossain *et al*. (2009). Kumar *et al*. (2005) reported that increase in the rate of photosynthesis is due to increased stomatal aperture, which facilitate more CO_2 conductance. Pierik *et al.* (2006) reviewed that enhanced stomatal conductance due to different concentrations stimulated photosynthesis. Cobalt at lower concentrations increased stomatal conductance, thereby increasing the rate of photosynthesis (Jaleel *et al*., 2009).

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 2). At vegetative stage, AL 1593 genotype had highest photochemical efficiency (0.726), while minimum was recorded in AL 1702 (0.670). While at flowering stage, AL 1578 recorded maximum photochemical activity (0.786) in all the treatments including control. Among the treatments, E_1V (0.784) followed by E_2V (0.782) recorded

Table 2 : 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				

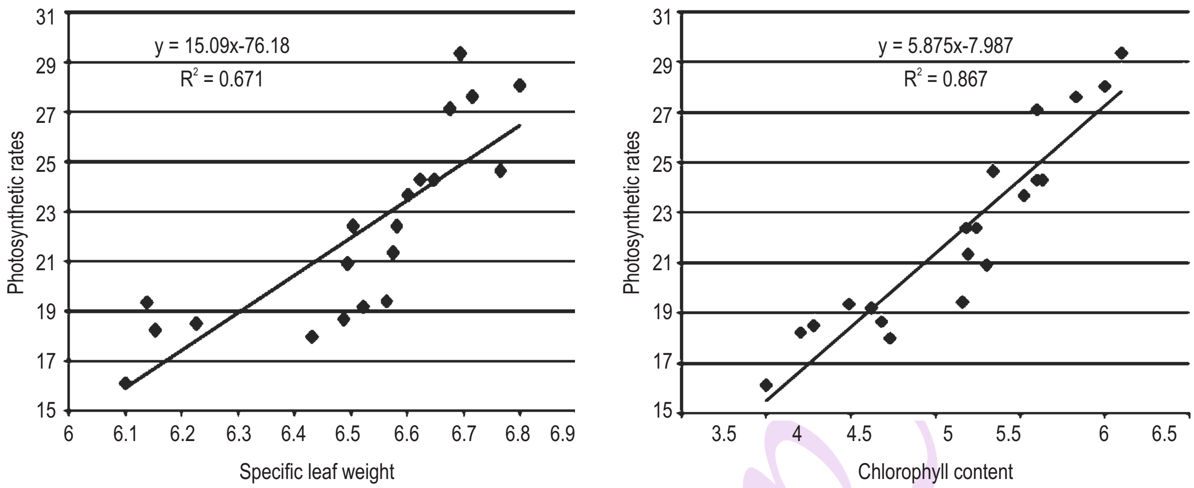


Fig. 2 : Effect of SLW and chlorophyll content at flowering on photosynthetic rate

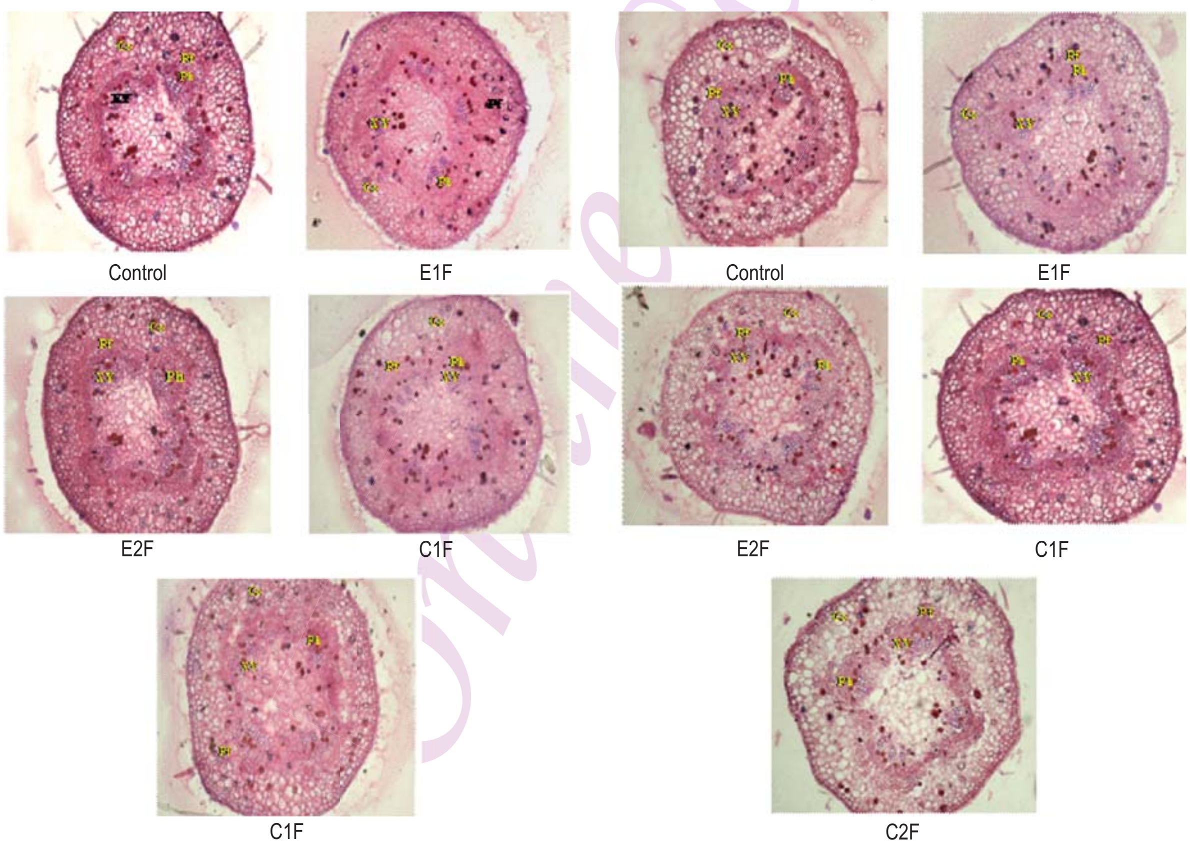


Fig. 3 : Transverse sections of pedicel of flower of pigeonpea genotype AL 1556 (a) and AL 201 (b) (XY=Xylem, Ph= Phloem, Pf= Phloem fibers, Co= Cortex)

Table 3 : 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				

Table 4 : Effect of ethrel and cobalt chloride on the structure of pedicel of pigeonpea genotypes AL 1578 and AL 201

			Total area (in 10Xview)		
Diameter	**Cortex**	**Phloem fiber**	**Phloem**	**Xylem**	
AL1578					
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
AL201					
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

maximum photochemical activity. At post-flowering stage, decrease in photochemical efficiency was recorded in all the genotypes and treatments. A maximum decrease in photochemical efficiency occurred in control plants.

Pigments such as chlorophylls are required by plants to absorb sufficient amount of light for photosynthesis. During leaf development, the level of pigments in leaves increases to provide energy through photosynthesis. Chlorophyll is an important bio-constituent for productivity of crop plants. Chlorophyll catabolism is the first step of degeneration during senescence most of the 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 (Table3). At vegetative stage, genotype AL 1593 showed maximum chlorophyll content (3.26 mg g^{-1} f. wt.) followed by AL 1578 (3.10 mg

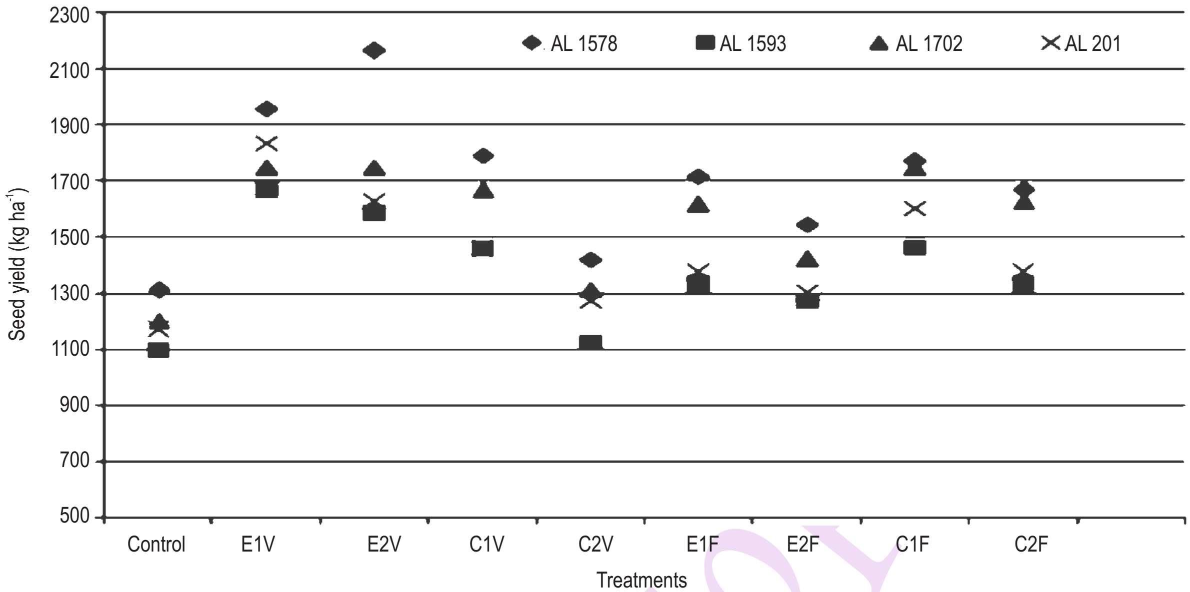


Fig. 4 : Effect of foliar application of ethrel and cobalt chloride on yield in pigeonpea genotypes

g^{-1} f. wt.) and minimum chlorophyll content was recorded in AL 1702 (2.94 mg g^{-1} fresh wt.). At flowering stage, maximum chlorophyll content was estimated in AL 1578 in all the treatments. Among the treatments E_1V recorded maximum chlorophyll content followed by E_2V and C_1V. A similar trend was seen at post- flowering stage.

Maximum chlorophyll content was seen at flowering stage which further decreased as the crop matured 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 highest in the end of the crop cycle, which reinforces the fact that cytokinins inhibit degradation of chlorophyll on plants. Non-degradation of chlorophyll on the tissue maintains them photosynthetically active, thus it provided larger quantity of organic matter to 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.

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. Relationship between specific leaf weight (SLW) and chlorophyll content with photosynthesis (Fig. 2) revealed 87 % variation in the rate of photosynthesis which 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 for yield and photosynthesis and 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).

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 4).The diameter of the pedicel increased in all the treatments as compared to control. The area of the main translocatory tissue *i.e.* phloem increased significantly in all the treatments. Due to increase in area of translocatory tissues in flower pedicel, more photosynthates were translocated towards sink which led to maximum retention of flowers and hence, highest yield in treated plants (Fig. 4). Seed yield was maximum in E_1V treatment followed by E_2V treatment and was found to be minimum in control plants. 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. Among genotypes AL 1578 had maximum seed yield followed by AL 1702 and minimum seed yield was found in AL 1593. Different treatments when applied exogenously at specific stage has remarkable effect in manipulating source-sink relationship and hence, seed yield.

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