ISSN 2347-2375

UNIQUE JOURNAL OF AYURVEDIC AND HERBAL MEDICINES

Available online: www.ujconline.net

Research Article

KEY APPROACHES OF PLANT GROWTH REGULATOR APPLICATION IN SEARCHING OF BEST TIME FOR ENHANCING NITROGEN FIXATION CAPACITY OF CHICKPEA CULTIVAR DCP 92-3

Mohd Mazid[1*], Nooris Naqvi[2]

[1]Advanced Plant Physiology Section, Department of Botany, AMU, Aligarh, India
[2]Environmental Science Division, Bareilly College, Rohilkhand University-Bareilly, India

Received 29-08-2014; Revised 27-09-2014; Accepted 25-10-2014

*Corresponding Author: **Mohd Mazid**
Advanced Plant Physiology Section, Department of Botany, AMU, Aligarh-202002

ABSTRACT

A pot experiment was conducted at Department of Botany, Aligarh Muslim University, Aligarh in the *'rabi'* season of 15th October, 2011 and harvest on the 30th March, 2012 to evaluate the effects of four levels of gibberellic acid (GA_3) spray (0, 10^{-7},10^{-6} and 10^{-5}M GA_3) at six spray stages of phenological development (60, 70, 80, 60-70, 70-80 and 60-70-80 days after sowing, DAS) on growth parameters viz., shoot length, root fresh weight per plant, nodule number per plant, nodule fresh weight per plant, nodule dry weight per plant, biochemical characteristics like nitrate reductase (NR) activity, leghaemoglobin (Lb) content (Lb) in nodule and leaf nitrogen (N) content and yield attributes, i.e., seed number per pod and seed protein content of chickpea (*Cicer arietinum* L.). The potted plants were then analyzed at 90 and 100 DAS for growth and biochemical parameters and at harvest for yield attributes. All parameters were found to be significantly promoted by the application of GA_3, with greater induction being noted at 60-70 DAS with 10^{-6}MGA.

Keywords: Chickpea, Nitrate Reductase Activity, Root Dry Weight, Seed Protein Content.

INTRODUCTION

In Asia, there is a widening gap exists between food production and population growth. In addition to declining grain production in recent years, grain legumes yields have also decreased to a greater extent compared to change in Australia and Europe. Several recent studies have therefore concluded that although, India has the potential to feed itself, food production is getting inadequate and unsustainable continuously. Consequently, food legumes aid to the Asian continent has double in last 10 years. With this fact, pulses are well known as good rotational crops and have achieved important place in the Indian cropping system to buildup sustainable agriculture[1].

Eleven primary pulses have been recognized by the Food and Agriculture Organization (FAO) of the United Nations at global level. These pulses include dry beans (common bean, *Phaseolus vulgaris* L.; lima bean, *Phaseolus lunatus* L.; tepary bean, *Phaseolus acutifolius* A. Gray; scarlet runner bean, *Phaseolus coccineus* L.; black gram, *Vigna mungo* L. Hepper; green gram, *Vigna radiata* L. Wilczek; moth bean, *Vigna aconitifolia* Jacq Marechal; rice bean,*Vigna umbellata* Thumb Ohwi and Ohashi and adzuki bean, *Vigna angularis* Willd Ohwi and Ohashi), dry broad bean (*Vicia faba* L.), dry peas (*Pisum* spp.) dry cowpea (*Vigna unguiculata* L. Walp), pigeon pea (*Cajanus cajan* L. Millspaugh), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik or *Lens esculenta* Moench), bambara groundnut (*Vigna subterranean* L. Verdc.), lupines (*Lupinus* spp.), common vetch (*Vicia sativa* L.) and minor pulses (lablab, *Lablab purpureus* L. Sweet; jack beans, *Canavalia ensiformis* L.DC.; sword bean, *Canavalia gladiate* Jacq. DC.; winged bean, *Psophocarpus teragonolobus* L. DC.; velvet bean, *Mucuna pruriens* L. DC. and yam bean, *Pachyrhizus erosus* L. Urb.)[2].

Among them, chickpea is one of the earliest pulse crops cultivated by humans. It is one of the most important pulse crops of India. It is grown only during *rabi* season as the climate is favorable for its growth and development. It is very nutritive and used as a protein adjunct to starchy diets and also playing a vital role in supplementing protein in vegetarian diet. It is given as preventive diet to antherosclerosis patients because of its rich phosphorus (P) content. Also, it is an ingredient of a Unani-anti-hypersensitive drug *'Ajmaloon'*. Whole germinated seeds are used as a prophylactic against

deficiency disorders, scurvy in particular in famine affected areas. A preparation of 25 per cent chickpea meal and 75 per cent groundnut meal used as a corrective in mal-nourished people and as a cure for Kwashiorkor and other protein deficiency diseases[3]. Moreover, its cultivation helps in sustaining soil fertility by fixing N up to 140 kilogram per hectare (kg/ha) per year[4].

Chickpea occupies the first position in India and third position at global level[5]. India is the largest producer as well as consumer of chickpea followed by Australia and Pakistan[5]. Chickpea is cultivated in arid and semi-arid areas around the world with an area of 11.1 million hectares adding 9.3 million tonnes of grains annually. Though chickpea is grown in our country in the largest area than other countries, but productivity at 911 kg/ha is much lower than developed countries[5]. There is limitation on increasing the acreage for cultivation, it is, therefore, highly logical to innovate ways that can improve the productivity. In all pulse crops, productivity is directly related to their N fixation ability.

To attain such goal, the use of GA_3 may play an important role as they are known to affect many facets of plant life[6-7]. GA_3 is a phytohormone that is needed in small quantities at low concentration to accelerate plant growth and development. So, favorable condition may be induced by applying GA_3 exogenously in proper concentration at a proper time in a specific crop by GA_3 (Khan et al., 2014). Also, GA_3 occupies a prominent position in mediating a variety of plant physiological processes[8], including activity of RuBPcase bunching of grapes, breaking of seed and bud dormancy, cell-wall plasticity, cell elongation, flowering, growth and yield of sugarcane, P_N, parthenocarpy, protein-synthesis, phloem-loading, stomatal aperture, senescence, stem-elongation, transcription of messenger (m)-RNA as well as it act as a florigen[9].

Moreover, it seems that there are a little investigation about the combined effect of timings and different concentrations on N-fixation and productivity of any pulse crop like chickpea. Considering the above fact, the present study was undertaken to known the N-fixation capability and subsequently protein content of seeds of chickpea under above mentioned situation of combined application of different doses with appropriate timings of crop maturity.

MATERIALS AND METHODS

A pot experiment was conducted during the *'rabi'* season of 2011-2012 on chickpea in Department of Botany, A M U, Aligarh. It is situated at 27.88 °N latitude 78.08°E longitude and 180 m average altitude with an area of 3700.4 sq km. Before sowing, the earthen pots of equal size were filled with the mixture of soil and FYM in the ratio of 3:2 at the rate of 6 kg /pot. The required number of pots was arranged according to a factorial randomized design. Authentic seeds of the chickpea cultivar, namely DCP 92-3 was delivered from the IIPR, Kanpur. The healthy seeds were soaked with double distilled water (DDW) for 3 h and then were surface sterilized with $HgCl_2$ and washing with DDW.

Prior to the foliar treatments, 100 milli-litre (Ml) stock solutions of GA_3 (SIGMA USA) at 10^{-3}M were prepared. Four concentrations of aqueous solution of GA_3 for foliar spray treatment, viz. 0, 10^{-7}, 10^{-6} and 10^{-5}M GA_3, constituted one variant and the six spray stages viz. 60, 70, 80, 60-70, 70-80 and 60-70-80 DAS, the other variant. A uniform recommended basal dose of 17.9 mg N+13.4 mg P/kg soil was applied to all pots with the half dose of N and full dose of P giving at the time of sowing and the remaining half dose of N after 50 DAS. Finally, four plants per pot were maintained. A water-sprayed control (Fw) was also included in the scheme of treatments.

Measurement of parameters

Growth parameters

Length of shoot on per plant basis was determined separately with the help of a metre scale. Weight of the fresh matter of root of each plant was determined separately with the help of an electronic balance. At the flowering stage, sample plants were collected from 1-2 cm linearly for the collection of nodules. The roots of the collected samples were washed carefully and all the nodules were separated manually for counting their number, fresh weight and later oven dried at 70˚C for 72 hours to record the dry weight of the nodules.

Physiological and Biochemical Characteristics

The enzyme, NR catalyses the reduction of NO_3^- to nitrite (NO_2^-).

$$NO_3^- + NADH + H^+ \xrightarrow{NR} NO_2^- + NAD + H_2O$$

The NR activity in fresh leaves was estimated by the method of Jaworski[10]. The leaves were cut into small pieces ($1cm^2$). Two hundred mg of these chopped leaves were weighed and transferred into plastic vials. To each vial, 2.5 ml phosphatic buffer of pH 7.5 and 0.5 ml KNO_3 solution were added followed by the addition of 2.5 ml 5% isopropanol. These vials were incubated in a BOD incubator for 2 h at 30±2˚C in the dark. 0.4 ml of incubated mixture was taken into test tube to which 0.3 ml each of sulphanilamide and nephthyl-ethylenediamine-dihydrochloride solutions were added. The test tube was left for 20 min. for maximum colure development. The mixture was diluted to 5 ml using DDW. The OD was recorded at 540 nm using the spectrophotometer.

N was estimated in dried powder of leaves obtained from each replicate. The sampled plant leaves were dried in an oven at 80˚C for 24 h. The dried leaves from each sample were finally powdered and then passed through a 72-mesh screen. For the estimation of these nutrients the leaf powder was first digested according to the standard technique described below.

100 mg oven dried powder of leaf material was transferred into a digestion tube to which 2 ml sulphuric acid (H_2SO_4) was added. The tube was then kept on a digestion assembly at 80˚C for about 2 h to allow the complete reduction of NO_3^- present in the plant material by the organic matter itself. Initially, dense white fumes were given off and then the content of the tube turned black. After cooling the tube for about 15 min, 0.5 ml 30% hydrogen peroxide (H_2O_2) was added drop by drop and the tube was heated again till the colour of the solution changed from black to light yellow. The digestion tube cooled for 10 min. and an additional amount (2-3 drops) of 30% H_2O_2 was added followed by gentle heating for about 15 min to get a clear and colourless solution. At this stage, care was taken in the addition of H_2O_2 because its

excess might oxidize ammonia in the absence of organic matter. The H_2O_2 digested material was diluted with DDW and transferred with three washings into a 100 ml volumetric flask and finally the volume was made up to the mark with DDW. The details of method employed for the estimation of N is given below.

Nitrogen

N was estimated according to the method of Lindner[11]. A 10 ml H_2SO_4-H_2O_2 digested material was taken into a 50 ml volumetric flask and the excess of the acid was neutralized by the addition of 2ml 2.5N NaOH. 1 ml 100% sodium silicate was added to prevent turbidity and finally, the volume was made up with DDW. Into a 10 ml graduated test tube, 5ml this solution was taken and 0.5 ml Nessler's reagent was added. The content of the test tube were allowed to stand for 5 min for maximum colour developed. The solution was transferred into a calorimetric tube and OD was read at 525 nm, using a blank on the spectrophotometer. N content was determined with the help of the standard curve and was expressed in terms of percentage on dry weight basis.

Lb occurs in the infected cells of legume root nodules. It facilitates O gas diffusion across the nodule into the N fixing bacteroids to support oxidation and at the same time ensures the O gas sensitive nitrogenase activity without damage. The Lb content in fresh nodules was estimated following the method described by Sadasivam and Manickam[12].

200 mg fresh nodules were mixed with 3 ml phosphate buffer (pH 7.4) and macerated with the help of pestle and mortar followed by filtration through two layers of cheese cloth. The nodule debris was discarded. The turbid reddish brown filterate was centrifuged at 10, 000 revolutions per minute (rpm) for 15 min. The supernatant was diluted to 5 ml with DDW. The extract (5 ml) was taken into a test tube followed by the addition of the same volume of alkaline pyridine reagent. The solution became greenish yellow due to the formation of haemochrome. The haemochrome was divided equally into the two test tubes. To the first test tube, 50 mg potassium hexacyanoferrate was added to oxidize the haemochrome and read at 539 nm on the spectrophotometer against a reagent blank. To the second test tube, 50 mg of sodium dithionate was added to reduce the haemochrome. The absorbance was read after 5 min at 556 nm against a reagent blank. The Lb content was calculated by the following formula:

$$\text{Lb content} = \frac{A556-A539\times 2D}{23.4} \text{m molg}^{-1} \text{ (nodule fresh mass)}$$

D is the initial dilution and A556 and A539 are the absorbances at 556 and 539 nm, respectively.

The calculation is based upon the equation

$$E = 23.4 \times 10^3 \text{ mol}^{-1}\text{cm}^{-1}.$$

Yield attributes

Seed number per pod

To assess the yield performance of the crop, the remaining two plants from each pot were harvested. The harvested plants were sun-dried in a net-house to prevent losses. After drying the crop, each sample was threshed individually. The seeds were utilized for assessing the other characteristics.

Seed protein content

The total protein content in the dry seeds was estimated by adopting the methodology of Lowry[13]. 50 mg oven dried seed powder was taken into a centrifuge tube and 5 ml 5% trichloroacetic acid was added to it. The solution was shaken throughly and allowed to stand for 30 min. at room temperature. The solution was centrifuged for 10 min at 4000 rpm and the supernatant was discarded. To the residue, 5ml 1N NaOH was added and mixed well. After 30 min, the solution was centrifuged and the supernatant was collected into a 50 ml volumetric flask. The residue was washed twice with 5 ml 1N NaOH and the washings were collected in the flask containing the supernatant. The volume of the flask was made up to the mark with 1N NaOH. 1ml of this NaOH extract was transferred to a 10 ml test tube and 5 ml of Reagent A (carbonate-copper sulphate solution) was added to it. The solution was mixed well and allowed to stand for 10 min at room temperature. Reagent B (diluted Folin's) at 0.5 ml was added rapidly with immediate mixing. After waiting for 30 min, the solution turned blue. The intensity of the blue coloured solution was measured with the spectrophotometer at 660 nm. A blank was run with each sample.

Statistical analysis

Full of data were analyzed statistically adopting the analysis of variance technique, according to Gomez and Gomez[14]. In applying the F test, the error due to replicates was also determined. When 'F' value was found to be significant at 5% level of probability, critical difference (CD) was calculated. The mean of the data of both the years were analyzed statistically using *SPPS-17* statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS

In this factorial randomized pot experiment, the effect of four concentrations of foliar spray of GA and of spray application stages, alone or in combination, was studied on the performance of chickpea cultivar DCP 92-3. The results (Tables 1-9; Figs.1) are summarized below.

Shoot length per plant

Of GA spray levels, 10^{-6}M GA ($F_{10^{-6}\text{ M GA}}$) proved best at both stages of sampling (90 and 100 DAS). The effect of this treatment was followed by that of $F_{10^{-7}\text{MGA}}$ at each stage. Spray level $F_{10^{-7}\text{M GA}}$ showed parity with $F_{10^{-5}\text{M GA}}$ in their effect at 90 DAS. $F_{10^{-6}\text{M GA}}$ gave 53.53 and 57.41% higher value at 90 and 100 DAS respectively than the water sprayed control (F_W). Among spray stages, foliar application at 60-70 DAS ($F_{60\text{-}70\ DAS}$) resulted in the maximum value at both sampling stages. Its effect was followed by that of $F_{60\ DAS}$ at both sampling stages and also by that of$F_{60\text{-}70\text{-}80\ DAS}$ at 100 DAS. Spray stage$F_{60\text{-}70\ DAS}$ gave 7.08 and 14.71% higher value at 90 and 100 DAS respectively than $F_{80\ DAS}$ which gave the minimum value. Interaction of $F_{10^{-6}\text{M GA}}$ with spray at 60-70 DAS ($F_{10^{-6}\text{M GA}} \times F_{60\text{-}70\ DAS}$) gave the maximum value at both sampling stages. However, its effect was at par with that of $F_{10^{-5}\text{M GA}} \times F_{70\ DAS}$ at each sampling stage and also with that of $F_{10^{-6}\text{M GA}} \times F_{60\ DAS}$ at 90 DAS and $F_{10^{-5}\text{M GA}} \times F_{60\text{-}70\ DAS}$ at 100 DAS. Interaction $F_{10^{-6}\text{M GA}} \times F_{60\text{-}70\ DAS}$ gave 65.40 and 83.42% higher value at 90 and 100 DAS respectively than the least value giving interaction $F_W \times F_{80\ DAS}$ (Table 1).

Table 1: Effect of concentrations of leaf-applied GA (C) and spray stages (F) on shoot length per plant (cm) of chickpea cultivar

Foliar spray stages (F_{DAS})	Foliar spray concentrations ($F_{M\ GA}$)				Mean
	F_W	$F_{10^{-7}\ MGA}$	$F_{10^{-6}\ MGA}$	$F_{10^{-5}\ MGA}$	
			90 DAS		
F_{60DAS}	46.70	69.32	72.15	70.13	64.58
F_{70DAS}	42.90	64.26	70.19	66.24	60.90
F_{80DAS}	44.80	66.40	69.20	66.97	61.84
$F_{60\text{-}70DAS}$	49.24	70.40	74.10	71.40	66.22
$F_{70\text{-}80DAS}$	46.10	65.29	69.82	68.23	62.36
$F_{60\text{-}70\text{-}80DAS}$	48.11	65.34	71.13	67.43	63.00
Mean	46.31	66.79	71.10	68.40	
C.D. at 5%		C = 1.658	F = 1.354	C x F = 3.316	
			100 DAS		
F_{60DAS}	47.20	65.25	69.32	68.20	62.49
F_{70DAS}	41.80	68.70	67.47	66.00	60.99
F_{80DAS}	39.80	67.70	68.70	54.44	57.59
$F_{60\text{-}70DAS}$	48.72	70.41	73.00	72.12	66.06
$F_{70\text{-}80DAS}$	38.70	64.53	65.72	63.40	58.09
$F_{60\text{-}70\text{-}80DAS}$	46.12	66.80	68.72	67.10	62.10
Mean	43.72	67.19	68.82	65.21	
C.D. at 5%		C = 1.616	F = 1.320	C x F = 3.232	

N.B.: A uniform basal dose of 40 kg N + 30 kg P_2O_5/ ha was applied to all pots.

Root fresh weight per plant

Spray concentration $F_{10^{-6}\ M\ GA}$ proved best at both stages of sampling and gave 83.34 and 77.60% higher value at 90 and 100 DAS respectively than F_W. Foliar spray stage $F_{60\text{-}70\ DAS}$ gave the maximum value at both sampling stages and its effect resulted in 4.23 and 6.87% higher value at 90 DAS and 100 DAS respectively than $F_{80\ DAS}$ which gave the minimum value. Interaction $F_{10^{-6}\ M\ GA} \times F_{60\text{-}70\ DAS}$ gave the maximum value at both sampling stages and gave 90.00 and 87.09% higher value at 90 and 100 DAS respectively than $F_W \times F_{80\ DAS}$ (Table 2).

Table 2: Effect of concentrations of leaf applied GA (C) and spray stages (F) on root fresh weight per plant (g) of chickpea cultivar DCP 92-3 at two growth stages (mean of four replicates)

Foliar spray stages (F_{DAS})	Foliar spray concentrations ($F_{M\ GA}$)				Mean
	F_W	$F_{10^{-7}\ M\ GA}$	$F_{10^{-6}\ M\ GA}$	$F_{10^{-5}\ MGA}$	
			90 DAS		
$F_{60\ DAS}$	1.21	2.15	2.19	2.16	1.93
$F_{70\ DAS}$	1.17	2.12	2.19	2.13	1.91
$F_{80\ DAS}$	1.20	2.10	2.13	2.11	1.89
$F_{60\text{-}70\ DAS}$	1.20	2.18	2.28	2.21	1.97
$F_{70\text{-}80\ DAS}$	1.19	2.11	2.18	2.12	1.97
$F_{60\text{-}70\text{-}80\ DAS}$	1.22	2.13	2.25	2.21	1.95
Mean	1.20	2.13	2.20	2.16	
C.D. at 5%		C = 0.051	F = 0.042	C x F = 0.103	
			100 DAS		
$F_{60\ DAS}$	1.23	2.13	2.26	2.24	1.96
$F_{70\ DAS}$	1.21	2.10	2.21	2.11	1.89
$F_{80\ DAS}$	1.24	2.10	2.11	2.11	1.89
$F_{60\text{-}70\ DAS}$	1.25	2.23	2.32	2.30	2.02
$F_{70\text{-}80\ DAS}$	1.24	2.10	2.20	2.20	1.93
$F_{60\text{-}70\text{-}80\ DAS}$	1.35	2.11	2.25	2.22	1.98
Mean	1.25	2.13	2.22	2.21	
C.D. at 5%		C = 0.052	F = 0.042	C x F = 0.104	

N.B.: A uniform basal dose of 40 kg N + 30 kg P_2O_5/ha was applied to all pots.

Nodule number per plant

Foliar level of $F_{10^{-6}\ M\ GA}$ proved best at both stages of sampling and gave 86.02 and 94.69% higher value at 90 and 100 DAS respectively than F_W. Foliar spray stage $F_{60\text{-}70\ DAS}$ proved best at both sampling stages and gave 13.64 and 6.82% higher value at 90 and 100 DAS respectively than $F_{80\ DAS}$. Interaction of $F_{10^{-6}\ M\ GA} \times F_{60\text{-}70\ DAS}$ gave the maximum value at both sampling stages. However, its effect was equalled by that of

$F_{10^{-6}\,M\,GA} \times F_{60\,DAS}$ at each sampling stage. Interaction $F_{10^{-6}\,M\,GA} \times F_{60\text{-}70\,DAS}$ gave 125 and 102.86% higher value at 90 and 100 DAS respectively than $F_W \times F_{80\,DAS}$ which gave the minimum value at 90 DAS (Table 3).

Table 3: Effect of concentrations of leaf applied GA (C) and spray stages (F) on nodule number per plant of chickpea cultivar DCP 92-3 at two growth stages (mean of four replicates)

Foliar spray stages (F_{DAS})	Foliar spray concentrations ($F_{M\,GA}$)				Mean
	F_W	$F_{10^{-7}\,M\,GA}$	$F_{10^{-6}\,M\,GA}$	$F_{10^{-5}\,M\,GA}$	
			90 DAS		
$F_{60\,DAS}$	36.00	67.00	71.00	65.00	59.75
$F_{70\,DAS}$	34.00	64.00	68.00	62.00	57.00
$F_{80\,DAS}$	32.00	63.00	65.00	60.00	55.00
$F_{60\text{-}70\,DAS}$	43.00	68.00	72.00	67.00	62.50
$F_{70\text{-}80\,DAS}$	38.00	66.00	67.00	64.00	58.75
$F_{60\text{-}70\text{-}80\,DAS}$	39.00	65.00	70.00	64.00	59.50
Mean	37.00	65.50	68.83	63.67	
C.D. at 5%		C = 37.00	F = 1.284	C x F = 3.144	
			100 DAS		
$F_{60\,DAS}$	32.00	64.00	69.00	63.00	57.00
$F_{70\,DAS}$	37.00	63.00	67.00	60.00	56.75
$F_{80\,DAS}$	35.00	62.00	65.00	58.00	55.00
$F_{60\text{-}70\,DAS}$	34.00	65.00	71.00	64.00	58.00
$F_{70\text{-}80\,DAS}$	31.00	62.00	66.00	61.00	55.00
$F_{60\text{-}70\text{-}80\,DAS}$	39.00	66.00	67.00	63.00	58.75
Mean	34.67	63.67	67.50	61.50	
C.D. at 5%		C = 1.524	F = 1.244	C x F = 3.048	

N.B.: A uniform basal dose of 40 kg N + 30 kg P_2O_5/ha was applied to all pots.

Nodule fresh weight per plant

Foliar level of $F_{10^{-6}\,M\,GA}$ proved best at both stages of sampling and gave 24.28 and 31.20% higher value at 90 and 100 DAS respectively than F_W. Foliar spray stage $F_{60\text{-}70\,DAS}$ gave the maximum value at both sampling stages and gave 4.90 and 25.40% higher value at 90 and 100 DAS respectively than F_{80DAS} which gave the minimum value. Interaction $F_{10^{-6}\,M\,GA} \times F_{60\text{-}70\,DAS}$ gave the maximum value at both sampling stages and gave 31.67 and 67.67% higher value at 90 and 100 DAS respectively than $F_W \times F_{80\,DAS}$ which gave the least value at 100 DAS (Table 4).

Table 4: Effect of concentrations of leaf applied GA (C) and spray stages (F) on nodule fresh weight per plant (g) of chickpea cultivar DCP 92-3 at two growth stages (mean of four replicates)

Foliar spray stages (F_{DAS})	Foliar spray concentrations ($F_{M\,GA}$)				Mean
	F_W	$F_{10^{-7}\,MGA}$	$F_{10^{-6}\,MGA}$	$F_{10^{-5}\,M\,GA}$	
			90 DAS		
$F_{60\,DAS}$	0.254	0.299	0.359	0.310	0.306
$F_{70\,DAS}$	0.283	0.315	0.337	0.327	0.316
$F_{80\,DAS}$	0.281	0.311	0.317	0.314	0.306
$F_{60\text{-}70\,DAS}$	0.279	0.315	0.370	0.319	0.321
$F_{70\text{-}80\,DAS}$	0.299	0.316	0.327	0.326	0.317
$F_{60\text{-}70\text{-}80\,DAS}$	0.259	0.309	0.349	0.315	0.308
Mean	0.276	0.311	0.343	0.319	
C.D. at 5%		C = 0.008	F = 0.007	C x F = 0.016	
			100 DAS		
$F_{60\,DAS}$	0.244	0.297	0.310	0.305	0.289
$F_{70\,DAS}$	0.231	0.287	0.302	0.292	0.278
$F_{80\,DAS}$	0.214	0.252	0.271	0.237	0.244
$F_{60\text{-}70\,DAS}$	0.244	0.315	0.359	0.320	0.306
$F_{70\text{-}80\,DAS}$	0.211	0.272	0.299	0.274	0.264
$F_{60\text{-}70\text{-}80\,DAS}$	0.253	0.291	0.319	0.307	0.293
Mean	0.234	0.286	0.307	0.289	
C.D. at 5%		C = 0.007	F = 0.006	C x F = 0.015	

N.B.: A uniform basal dose of 40 kg N + 30 kg P_2O_5/ha was applied to all pots.

Nodule dry weight per plant

GA spray level $F_{10^{-6}M\ GA}$ proved best at both stages of sampling and gave 138.89 and 138.76% higher value at 90 and 100 DAS respectively than F_W. Foliar spray stage $F_{60\text{-}70\ DAS}$ gave the maximum value at both sampling stages. Its effect was followed by that of $F_{60\ DAS}$ and $F_{60\text{-}70\text{-}80\ DAS}$ at each sampling stage. Foliar spray stage $F_{60\text{-}70\ DAS}$ gave 21.76 and 24.07% higher value at 90 and 100 DAS respectively than $F_{80\ DAS}$. Interaction $F_{10^{-6}MGA} \times F_{60\text{-}70\ DAS}$ gave the maximum value at both sampling stages and gave 184.06 and 191.30% higher value at 90 and 100 DAS respectively than $F_W \times F_{80\ DAS}$ which gave the least value (Table 5).

Table 5: Effect of concentrations of leaf applied GA (C) and spray stages (F) on nodule dry weight per plant (g) of chickpea cultivar DCP 92-3 at two growth stages (mean of four replicates)

Foliar spray stages (F_{DAS})	Foliar spray concentrations ($F_{M\ GA}$)				Mean
	F_W	$F_{10^{-7}M\ GA}$	$F_{10^{-6}M\ GA}$	$F_{10^{-5}M\ GA}$	
			90 DAS		
$F_{60\ DAS}$	0.137	0.347	0.359	0.352	0.299
$F_{70\ DAS}$	0.149	0.332	0.332	0.341	0.289
$F_{80\ DAS}$	0.138	0.299	0.311	0.301	0.262
$F_{60\text{-}70\ DAS}$	0.140	0.370	0.392	0.373	0.319
$F_{70\text{-}80\ DAS}$	0.150	0.323	0.327	0.325	0.281
$F_{60\text{-}70\text{-}80\ DAS}$	0.151	0.337	0.342	0.340	0.293
Mean	0.144	0.335	0.344	0.339	
C.D. at 5%		C = 0.008	F = 0.007	C x F = 0.016	
			100 DAS		
$F_{60\ DAS}$	0.147	0.345	0.372	0.350	0.304
$F_{70\ DAS}$	0.159	0.327	0.332	0.329	0.287
$F_{80\ DAS}$	0.138	0.310	0.319	0.311	0.270
$F_{60\text{-}70\ DAS}$	0.152	0.392	0.402	0.395	0.335
$F_{70\text{-}80\ DAS}$	0.144	0.316	0.327	0.319	0.277
$F_{60\text{-}70\text{-}80\ DAS}$	0.142	0.347	0.352	0.350	0.298
Mean	0.144	0.340	0.351	0.342	
C.D. at 5%		C = 0.008	F = 0.007	C x F = 0.016	

N.B.: A uniform basal dose of 40 kg N + 30 kg P_2O_5/ ha was applied to all pots.

Nitrate reductase activity

Of GA spray levels, $F_{10^{-6}M\ GA}$ proved best at both stages of sampling. Its effect was followed by that of $F_{10^{-5}M\ GA}$ at each sampling stage, with $F_{10^{-7}M\ GA}$ also following at 90 DAS. Spray concentration $F_{10^{-5}M\ GA}$ gave 28.69 and 21.23% higher value at 90 and 100 DAS respectively than F_W. Spray stage $F_{60\text{-}70\ DAS}$ gave the maximum value at both sampling stages. However, its effect was equalled by that of $F_{60\ DAS}$ at 90 DAS and by that of $F_{60\text{-}70\text{-}80\ DAS}$ at 100 DAS. Spray stage $F_{60\text{-}70\ DAS}$ gave 7.43 and 6.98% higher value at 90 and 100 DAS respectively than $F_{80\ DAS}$. Interaction $F_{10^{-6}M\ GA} \times F_{60\text{-}70\ DAS}$ gave the maximum value at both sampling stages. However, its effect was at par with that of $F_{10^{-6}M\ GA} \times F_{60\ DAS}$ and $F_{10^{-7}M\ GA} \times F_{60\text{-}70\ DAS}$ at 90 DAS and with that of $F_{10^{-6}M\ GA} \times F_{60\text{-}70\text{-}80\ DAS}$ at 100 DAS and gave 38.42 and 29.57% higher value at 90 and 100 DAS respectively than $F_W \times F_{80\ DAS}$ which gave the minimum value (Table 6).

Table 6: Effect of concentrations of leaf applied GA (C) and spray stages (F) on nitrate reductase activity (n mol $NO_2^-kg^{-1}$ (leaf fresh mass) s^{-1}] of chickpea cultivar DCP 92-3 at two growth stages (mean of four replicates)

Foliar spray stages (F_{DAS})	Foliar spray concentrations ($F_{M\ GA}$)				Mean
	F_W	$F_{10^{-7}M\ GA}$	$F_{10^{-6}M\ GA}$	$F_{10^{-5}MGA}$	
			90 DAS		
$F_{60\ DAS}$	83.58	105.31	112.85	109.12	102.70
$F_{70\ DAS}$	84.00	93.16	108.25	99.54	96.23
$F_{80\ DAS}$	82.69	96.97	104.83	98.92	95.85
$F_{60\text{-}70\ DAS}$	86.06	111.03	114.47	103.36	103.73
$F_{70\text{-}80\ DAS}$	87.56	90.91	105.11	96.97	95.13
$F_{60\text{-}70\text{-}80\ DAS}$	88.09	99.64	110.62	99.27	99.40
Mean	85.33	99.50	109.34	101.19	
C.D. at 5%		C = 15.393	F = 2.094	C x F = 5.131	
			100 DAS		
$F_{60\ DAS}$	104.20	112.52	127.20	118.89	115.70
$F_{70\ DAS}$	105.11	111.15	122.52	119.20	114.49
$F_{80\ DAS}$	106.13	111.03	122.69	114.91	113.69

$F_{60\text{-}70\ DAS}$	110.39	111.05	137.53	122.53	121.62
$F_{70\text{-}80\ DAS}$	102.55	113.65	123.08	118.64	114.48
$F_{60\text{-}70\text{-}80\ DAS}$	106.31	111.97	136.41	130.31	121.25
Mean	105.78	112.72	128.23	120.74	
C.D. at 5%		C = 2.991	F = 2.492	C x F = 5.981	

N.B.: A uniform basal dose of 40 kg N + 30 kg P_2O_5 /ha was applied to all pots.

Nitrogen content

Spray level $F_{10^{-6}\ M\ GA}$ proved best at both stages of sampling and gave 12.85 and 12.43% higher value at 90 and 100 DAS respectively than F_W. Spray stage $F_{60\text{-}70\ DAS}$ gave the maximum value at both sampling stages. Its effect was, however, equalled by that of $F_{60\ DAS}$ at 90 DAS and by that of $F_{70\ DAS}$ at 100 DAS. Spray stage $F_{60\text{-}70\ DAS}$ gave 8.07 and 10.53 % higher value at 90 and 100 DAS respectively than $F_{80\ DAS}$. Interaction $F_{10^{-6}\ M\ GA} \times F_{60\text{-}70\ DAS}$ gave the maximum value at both sampling stages. Its effect was, however, at par with that of $F_{10^{-6}\ M\ GA} \times F_{70\ DAS}$ at 90 DAS and with that of $F_{10^{-6}\ M\ GA} \times F_{60\text{-}70\text{-}80\ DAS}$ at 100 DAS and gave 3.98 and 26.63% higher value at 90 and 100 DAS respectively than $F_W \times F_{80\ DAS}$ (Table 7).

Table 7: Effect of concentrations of leaf applied GA (C) and spray stages (F) on leaf nitrogen content (%) of chickpea cultivar DCP 92-3 at two growth stages (mean of four replicates)

Foliar spray stages (F_{DAS})	Foliar spray concentrations ($F_{M\ GA}$)				Mean
	F_W	$F_{10^{-7}\ MGA}$	$F_{10^{-6}\ M\ GA}$	$F_{10^{-5}\ M\ GA}$	
			90 DAS		
$F_{60\ DAS}$	3.421	3.721	3.784	3.789	3.679
$F_{70\ DAS}$	3.321	3.754	3.825	3.869	3.647
$F_{80\ DAS}$	3,324	3.521	3.720	3.400	3.466
$F_{60\text{-}70\ DAS}$	3.439	3.825	3.997	3.724	3.746
$F_{70\text{-}80\ DAS}$	3.390	3.625	3.794	3.574	3.596
$F_{60\text{-}70\text{-}80\ DAS}$	3.420	3.520	3.689	3.629	3.565
Mean	3.369	3.661	3.802	3.634	
C.D. at 5%		C = 0.093	F = 0.076	C x F = 0.186	
			100 DAS		
$F_{60\ DAS}$	3.743	3.927	3.930	3.840	3.860
$F_{70\ DAS}$	3.780	4.000	3.104	3.941	3.958
$F_{80\ DAS}$	3.349	3.710	3.731	3.640	3.610
$F_{60\text{-}70\ DAS}$	3.624	4.104	4.251	3.990	3.990
$F_{70\text{-}80\ DAS}$	3.497	3.992	4.100	3.985	3.892
$F_{60\text{-}70\text{-}80\ DAS}$	3.584	3.982	4.143	3.842	3.888
Mean	3.596	3.953	4,043	3.873	
C.D. at 5%		C = 0.099	F = 0.081	C x F = 0.199	

N.B.: A uniform basal dose of 40 kg N + 30 kg P_2O_5 /ha was applied to all pots.

Leghaemoglobin content

Of GA spray levels, $F_{10^{-6}\ M\ GA}$ proved best at both stages of sampling and gave 172.34 and 148.41% higher value at 90 and 100 DAS respectively than F_W. Foliar spray stage $F_{60\text{-}70\ DAS}$ proved best at 90 DAS and $F_{60\text{-}70\text{-}80\ DAS}$, at 100 DAS. The effect of spray stage $F_{60\text{-}70\ DAS}$ was followed by that of F_{60DAS} and $F_{60\text{-}70\text{-}80\ DAS}$ at 90 DAS and of spray stage $F_{60\text{-}70\text{-}80\ DAS}$, by that of $F_{60\text{-}70\ DAS}$ and F_{60DAS} at 100 DAS. Spray stage $F_{60\text{-}70\ DAS}$ gave 18.81 and 15.87 % higher value at 90 and 100 DAS respectively than $F_{80\ DAS}$ which gave the minimum value. Interaction $F_{10^{-6}\ M\ GA} \times F_{60\text{-}70\ DAS}$ gave the maximum value at both sampling stages and gave 220.45 and 175.00% higher value at 90 and 100 DAS respectively than $F_W \times F_{80\ DAS}$ which gave the least value (Table 8).

Seed number per pod

Of GA spray levels, $F_{10^{-6}\ M\ GA}$ proved best and gave 210.67% higher value than F_W. Foliar spray stage $F_{60\ DAS}$ gave the maximum value. Its effect was followed by that of spray stage $F_{70\text{-}80\ DAS}$ and gave 33.58% higher value than the least value giving spray stage $F_{70\ DAS}$. Interaction $F_{10^{-6}\ M\ GA} \times F_{70\text{-}80\ DAS}$ gave the maximum value and gave 450% higher value than the least value giving interaction $F_W \times F_{70\ DAS}$ (Table 9).

Seed protein content

The effect of spray levels of GA and of spray application stages, alone as well in combination, was significant on seed protein content. Of GA spray levels, $F_{10^{-6}\ M\ GA}$ proved best. Its effect was followed by that of $F_{10^{-7}\ M\ GA}$ and $F_{10^{-5}\ M\ GA}$. Treatment $F_{10^{-6}\ M\ GA}$ gave 24.42% higher value than F_W. Foliar spray stage $F_{60\ DAS}$ gave the maximum value. Its effect was followed by that of spray stage $F_{70\text{-}80\ DAS}$, $F_{60\text{-}70\ DAS}$ and $F_{60\text{-}70\text{-}80\ DAS}$. Spray stage $F_{60\text{-}70\ DAS}$ gave 7.05% higher value than the least value giving spray stage F_{80DAS}. Interaction $F_{10^{-7}\ M\ GA} \times F_{60\ DAS}$ gave the maximum value. Its effect was followed by that of $F_{10^{-6}\ M\ GA} \times F_{60\text{-}70DAS}$, $F_{10^{-6}\ MGA} \times F_{70\text{-}80DAS}$ and $F_{10^{-6}\ M\ GA} \times F_{60\text{-}70\text{-}80\ DAS}$. Interaction $F_{10^{-6}\ M\ GA} \times F_{60\text{-}70\ DAS}$ gave 48.57% higher value than $F_W \times F_{80\ DAS}$ which gave the minimum value (Fig. 1).

Table 8: Effect of concentrations of leaf applied GA (C) and spray stages (F) on leghaemoglobin content (m molg^{-1} (nodule fresh mass)] of chickpea cultivar DCP 92-3 at two growth stages (mean of four replicates)

Foliar spray stages (F_{DAS})	Foliar spray concentrations ($F_{M\ GA}$)				Mean
	F_W	$F_{10^{-7}\ M\ GA}$	$F_{10^{-6}\ M\ GA}$	$F_{10^{-5}\ M\ GA}$	
			90 DAS		
$F_{60\ DAS}$	0.149	0.377	0.400	0.382	0.327
$F_{70\ DAS}$	0.140	0.331	0.372	0.345	0.297
$F_{80\ DAS}$	0.132	0.321	0.353	0.343	0.287
$F_{60\text{-}70\ DAS}$	0.152	0.392	0.423	0.397	0.341
$F_{70\text{-}80\ DAS}$	0.138	0.349	0.362	0.357	0.302
$F_{60\text{-}70\text{-}80\ DAS}$	0.132	0.371	0.392	0.389	0.321
Mean	0.141	0.357	0.384	0.369	
C.D. at 5%		C = 0.009	F = 0.007	C x F = 0.017	
			100 DAS		
$F_{60\ DAS}$	0.147	0.362	0.409	0.397	0.329
$F_{70\ DAS}$	0.140	0.372	0.400	0.391	0.326
$F_{80\ DAS}$	0.156	0.341	0.350	0.337	0.296
$F_{60\text{-}70\ DAS}$	0.148	0.382	0.429	0.379	0.335
$F_{70\text{-}80\ DAS}$	0.180	0.321	0.347	0.340	0.297
$F_{60\text{-}70\text{-}80\ DAS}$	0.168	0.397	0.403	0.405	0.297
Mean	0.157	0.363	0.390	0.375	
C.D. at 5%		C = 0.009	F = 0.007	C x F = 0.018	

N.B.: A uniform basal dose of 40 kg N + 30 kg P_2O_5 /ha was applied to all pots.

Table 9: Effect of concentrations of leaf-applied GA (C) and spray stages (F) on seed number per pod of chickpea cultivar DCP 92-3 at harvest (mean of four replicates)

Foliar spray stages (F_{DAS})	Foliar spray concentrations ($F_{M\ GA}$)				Mean
	F_W	$F_{10^{-7}\ M\ GA}$	$F_{10^{-6}\ M\ GA}$	$F_{10^{-5}\ M\ GA}$	
$F_{60\ DAS}$	1.00	1.75	2.25	2.00	1.75
$F_{70\ DAS}$	0.50	1.00	2.00	1.75	1.31
$F_{80\ DAS}$	1.00	1.75	2.25	1.50	1.63
$F_{60\text{-}70\ DAS}$	0.50	1.00	2.50	1.75	1.37
$F_{70\text{-}80\ DAS}$	0.75	1.25	2.75	1.50	1.68
$F_{60\text{-}70\text{-}80\ DAS}$	0.75	1.25	2.25	2.00	1.56
Mean	0.75	1.33	2.33	1.75	
C.D. at 5%		C = 0.043	F = 0.035	C x F = 0.087	

N.B.: A uniform basal dose of 40 kg N + 30 kg P_2O_5/ha was applied to all pots.

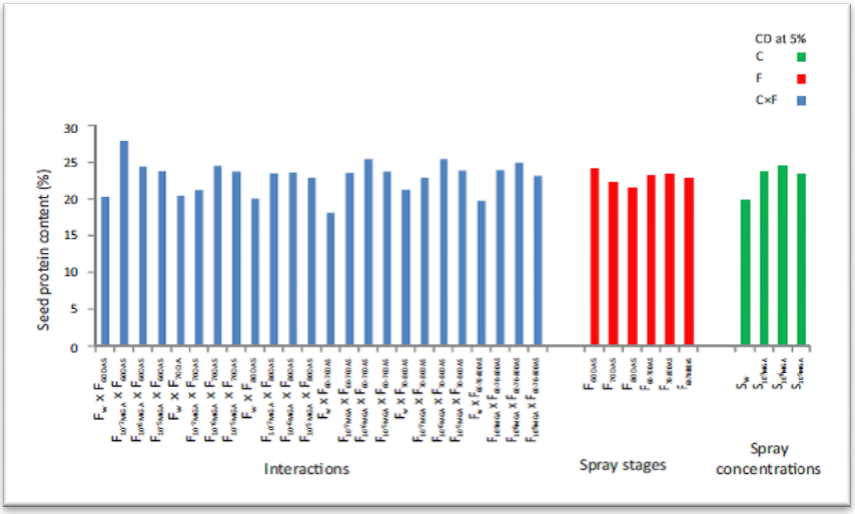

Figure 1: Effect of concentrations of leaf-applied GA and spray stages on seed protein content of cultivar DCP 92-3 of chickpea

DISCUSSION

The vegetative and reproductive growth of plants depends mainly on their ability to fix C in organs having chloroplasts followed by the utilization of the photosynthates for sink organs. The major metabolic processes influenced in the hormone treated plants are the C-assimilation, distribution of metabolites into the plant organs, and subsequent utilization by biosynthetic pathways[15]. GA_3 mediating a variety of plant physiological processes viz., seed germination, leaf expansion, flower and fruit set, dry matter production, photosynthesis, translocation of food material and synthesis of hydrolytic enzymes[8]. As the C fixing ability of plants is influenced by GA_3 among other factors, and it also affects production of dry matter and partitioning of photosynthates[16]. Moreover, the improvement of a crop depends to a larger extent on the genetic variability as it provides us the raw materials for selection of better genotypes. Little natural variability is found in chickpea for conspicuous morphological and physiological characters. GA_3 serve manifold growth related functions in plants by enhancing replication, transcription and different enzymatic systems[17].

The enhancing effect of foliar application of GA with10^{-6}M at 60-70 DAS over the water-sprayed control as well as its superior effect among the various other levels on shoot length of chickpea cultivar, DCP 92-3 can be traced to its various roles in plants. The application of GA improves absorption and use efficiency of nutrients[18], activity of enzymes[19], cell division and cell enlargement[9], chlorophyll content, elongation of internode[8], membrane permeability[20-9], P_N, nucleic acid and protein synthesis[8], and transport of photosynthates[21]. Foliar application of GA could have led to the observed improvement in shoot length per plant of the treated plants (Tables 1). GA treatment produced marked phenotypic changes such as pale yellow leaves showing serrated white midrib, long internodes, and elongated plants as compared to untreated plants. Earlier studies have reported also that GA_3 as foliar spray on transplanted cutting increased plant height.

Moreover, GA_3 foliar spray increased plant height and leaf length[22]. An increase in growth parameters like shoot and root lengths, fresh and dry weights in plants sprayed with GA_3 in accordance with the known fact that exogenous application of PGRs evoke the intrinsic genetic potential of the plant causing increase in elongation of internodes as a consequence of cell division and cell wall extensibility. Thus, (Tables 1) establish beyond doubt the superiority of foliar application of GA_3 over water-sprayed control. These results broadly corroborate the findings of earlier workers including[23-25].

The growth improving effect of foliar treatment at 60 and 70 DAS with 10^{-6}M GA over their respective water treated control on plant height, root fresh weight per plant grown with the recommended basal dose of N and P could be explained on the basis of its roles mentioned in introduction and the fact that the supply of GA_3 by foliar application would more than compensate the 'hidden hunger' of growing crops for GA_3. Improvement in shoot length and root fresh weight of chickpea (Tables 1-2) would have contributed in improving the ability of treated plants for nodule and biomass production. This is manifested in the observed improvement in their fresh and dry weight is further confirmed by correlation studies (r=0.934). Moreover, it contributes towards enhancing the capacity of the treated plants for biomass production as reflected in shoot and root dry weight of the plants. This enhancement could be the results of increased uptake of nutrients, enhanced photosynthesis and improved translocation of photosynthates and other metabolites to the reproductive parts. This sustained increase in the above mentioned parameters of the treated plants which is expected to culminate the maximization of seed protein content.

The augmenting effect of leaf-applied GA over the water-sprayed control as also its superior effect at 10^{-6}M on NR activity studied at 90 and 100 DAS is worth mentioning. The increase in NR activity can be attributes to the hormone-induced increase in transcription and/or translation of the gene that codes for CA[26-22] and to its role in enhancing the permeability of membranes and absorption of nutrients[27-28]. These results are also in accordance with the data of earlier workers[29, 26]. In turn, the CO_2 might have been acted upon by CA. Finally, CO_2 could be reduced by Rubisco in the chloroplast stroma. A probable reason for the enhancement of CA activity due to application of foliar spray of GA_3 might be the *de novo* synthesis of CA, which involves translation/ transcription of the associated DNA. Enhancement in the CA activity in treated plants might have responsible for the enhanced rate of CO_2 fixation and hence have resulted in significant increase in fresh & dry weight of the treated plants.

Needless to say that GA_3 may also be attributed, as for growth characters, to its roles on one hand and compensation of the 'hidden hunger' for GA_3 by its foliar application on the other. These results also corroborate the findings of Shah[29] on NR activity. Enhanced rate of NR activity would have resulted in improving the P_N and gs (data not published) of treated plants. Likewise, increased NR activity might be responsible for increasing biosynthesis of Chl (data not published) that in turn would have improved P_N of treated plants. Needless to say that improvement in N content would also have enhanced content of Chl. Enhancement in leaf-nutrients, particularly N due to GA_3 application could be attributed to the compositional or chemical change in plants leading to alterations in N concentration. Presumably, increased uptake of nutrients enhanced photosynthesis and improved translocation of photosynthates and other metabolites to the sinks that might have contributed to the improved yield of GA_3 treated plants. These findings are in accordance with data on GA_3 effects reported regarding plant nutrient elements.

Furthermore, acquisition and assimilation of N is a fundamental process that is essential for the growth and development of plants. N is available to plants mainly in the form of nitrate. The nitrate taken up by the plants is first reduced to NH_3 by the enzyme namely, NR and nitrite reductase. N assimilation enzyme which are crucial for plant growth and also provide effective targets for herbicide development. N-supply in the form of NH_3 or urea led to a significant decrease in the NR activity. In contrast, an increase in the supply of nitrate elicited an increase in NR activity, which was further enhanced when equal concentrations of both nitrate and ammonium ions were supplied.

No doubt, source-sink relationship is significantly influenced by hormones including GA_3 hence distribution of C^{14} assimilates to roots was analysed. The biomass accumulation depends on photosynthetic efficiency that is reduced by GA_3 treatment. This is an imperative association between inter-organ assimilation transports, particularly shoot and root partitioning of metabolites, and biomass production. Moreover, GA_3 enhances metabolic activity within pathways leading to accumulation of metabolites, e.g., protein, essential oil. Ontogenic studies in Chickpea have revealed that young rather than old leaves efficiently utilize CO_2 assimilates for metabolite production. Now it is unequivocally proved that GA_3 treatment on whole plant produced phenotypic response in total biomass production with positive response in the content of total seed protein content.

The increase in the seed number per pod resulting from the foliar application of GA_3 in comparison with the water-sprayed control as well as its superior effect at 10^{-6} M on this parameter, is worth mentioning. The increase in the above yield attribute may be traced to its various roles mentioned in introduction leading to observed higher values for growth characters and, physiological and biochemical parameters of treated plants. Moreover, it mediates differentiation[30] leading to enhanced number of flowers which develop into pods. As stated that it plays role in cell division and cell enlargement[31-32], resulting in proper development of under-developed pods especially at the terminal end of branches; NR supplying sufficient C skeleton; and membrane permeability and transport of photosynthates[33] favouring partitioning, hence, higher values for the yield parameters of treated plants. These results broadly corroborate the findings of Akter[34].

Also, comprehensive studies in grasses show that GA_3 play a role as a florigen[35]. The augmenting effect of foliar treatment with 10^{-6}M GA for 60-70 DAS over water-spray treatment on seeds per pod is understandable. GA_3 might have increased the translocation of assimilates to the reproductive organ which resulted in the maximum number of pods per plant uo to a certain levels of GA_3 application[36]. Moreover, GA_3 might be involved in formation of seeds in pods and their optimum nourishments have resulted in less number of aborted seeds and thus maximized the survival of fertile seeds/pods in chickpea[37].

In addition, this may be due to its roles mentioned above for improving these parameters and offset of the 'hidden hunger' for GA by its foliar application. The increased yield attributing parameters of treated plants, particularly seed number per pods per is likely to have contributed to the improved seed yield (unpublished data). This proposition is confirmed by correlation studies also wherein various yield characters may be noted to the positively and significantly correlated with seed yield. The observed increase in seed protein content due to foliar application of GA (Fig. 1) is not surprising. An improvement in protein synthesis may result from the foliar application of GA[38], hence higher value for seed protein content. These results broadly corroborate with the findings of Khafagy[39].

It was tempting to suppose that the addition of 10^{-6}M GA_3 at 60-70 DAS might contribute to some mechanism for stimulating the best circumstances for better N-fixation which subsequently results in greater enhancement in protein biosynthetic pathway in chickpea.

CONCLUSION

Therefore, it may be suggested that the combined foliar spray of (10^{-6}MGA_3 at 60-70 DAS) was highly effective for better N-fixation and good protein yield and improved the overall performance of the crop. It proved considerably important for enhancing photosynthesis, crop productivity and seed yield, activities of NR, CA and protein content of seeds. Thus, (10^{-6}MGA_3 at 60-70 DAS) concentration along with this specific duration, in the form of foliar spray, might presumably be recommended for maximizing the productivity and quality of *Cicer arietinum* L.

ACKNOWLEDGEMENTS

We are grateful to the Chairman, Department of Botany, Aligarh Muslim University, Aligarh, for providing research facilities. We are also grateful to Prof D.K. Saxena for his critical comments and valuable suggestions with regard to the preparation of the manuscript.

REFERENCES

1. Mazid M, Mohammad F. Environmental constraints responsible for reduction in productivity of chickpea: Revisit an old problem. Int. J. Envt. Eng. Manage. 2012; 3 (5): 32-35.
2. Khan TA, Mazid M, & Mohammad F. Status of secondary plant products under abiotic stress: an overview. Journal of Stress Physiology & Biochemistry. 2011; 7 (2): 75-98.
3. Nene, YL. Indian pulses through the millennia. Asian Agric - History 10: 179-202.
4. Anonymous (1992). The Wealth of India. Vol.III. C.S.I.R., New Delhi. 2006; pp. 340-349.
5. FAO. http://faostat.fao.org/default.aspx December, 2010 to March, 2011.
6. Rupela, O. Nodulation and nitrogen fixation in chickpea. In: The Chickpea. M. Saxena and K.B. Singh (eds.). CAB International, Wallingford, UK, 1987; pp. 191-206.
7. Gana AS. The role of synthetic growth hormones in crop multiplication and improvement. African J. Biotechnol. 2010; 10:10330-10334.
8. Mazid M. Seed priming application of gibberellic acid on growth, biochemical, yield and protein status of Chickpea (Cicer arietinum L. cv. DCP 92-3). International Journal of Genetic Engineering and Biotechnology. 2014; 5(1): 17-22.
9. Khan F, Mazid M, Khan TA, Patel HK, Rajiv R. Chowdhury. Plant derived pesticides in control of Lepidopterian insects: Dictum and Directions. Res. J. Bio.2014; 2 (1): 1-10.
10. Tiwari DK, Pandey P, Giri SP, Dwivedi JL. Effect of GA_3 and other plant growth regulators on hybrid rice seed production. Asian J. Plant Sci. 2011; 10: 133-139.

11. Taiz L, Zeiger E. Plant Physiology. 5th Ed. Sinauer Associates, Inc., 2010; Sunderland, MA, USA.
12. Jaworski EG. Nitrate reductase assay in intact plant tissues. Biochem. Biophys. Res. Commun. 1971; 43: 1274-1279.
13. Lindner RC. Rapid analytical methods for some of the more common inorganic constituents of plant tissues. Plant Physiol. 1944; 19: 76-89.
14. Sadasivan S, Manickam A. Biochemical Methods. 3ed Ed., New Age International, 2008, New Delhi.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem.1951; 193: 265-275.
16. Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research. 2nd Ed. J. Wiley and Sons, 1984, New York.
17. Verpoorte R, Vander Heijden R, Moreno PRH. Biosynthesis of terpene indole alkaloids in *Cathranthus roseus* cells. In: Cordell, G.A (ed.): The alkaloids. 1997; Vol 49: pp, 221-299.Academic Press, New York.
18. Kharche PV, Kubde KJ, Solunke PS. Effect of phosphorus, sulphur and PSB on quality components and nutrient uptake in chickpea. Ann. Plant Physiol. 2006; 20: 78-81.
19. Khan TA, Mazid M, & Mohammad F. Status of secondary plant products under abiotic stress: an overview. Journal of Stress Physiology & Biochemistry. 2011; 7 (2), 75-98.
20. Sandahya D, Kathuria E, Kakralya BL, Malik CP. Influence of plant growth regulators on photosynthesis in moong bean subjected to water-stress. Indian J. Plant Physiol. 2012; 17: 241-245.
21. Yuan L, Xu DQ. Stimulation effect of gibberellic acid short-term treatment on leaf photosynthesis related to the increase in rubisco content in broad bean and soybean. Photosynth. Res. 2001; 68: 39-47.
22. Bhandal IS, Singh R, Malik CP. Effect of some growth substances and phenolic compounds on membrane permeability in beet root. Phyton, 1985; 25: 177-184.
23. Ouzounidou G, Ilias I. (2005). Hormone-induced protection of sunflower photosynthetic apparatus against copper toxicity. Biol. Plant. 49: 223-228.
24. Iqbal HF, Tahir A, Khalid MN, I-ul-Haq Ahmad AN. Response of chickpea (*Cicer arietinum* L.) growth towards the foliar application of gibberellic acid at different growth stages. Pak. J. biol. Sci. 2001; 4: 433-434.
25. Yadav RM, Bharud RW. Response of Kabuli chickpea to the foliar application of growth substances. Mysore J. Agri. Sci. 2006; 40: 134-137.
26. Wood A, Paleg LG. The influence of gibberellic acid on the permeability of model membrane systems. Plant Physiol. 1972; 50: 103-108.
27. Mazid M, Khan TA, Mohammad F. Role of nitrate reductase in nitrogen fixation under photosynthetic regulation. World. J. Pharmaceu. Res. 2012; 1 (3): 386-414.
28. Khan TA, Mazid M, & Mohammad F. Potential of ascorbic acid against oxidative burst in plants under biotic stress: A Review. Journal of Industrial Research & Technology. 2011; 1 (1): 01-05.
29. Shah SH. Physiological effects of pre-sowing seed treatment with gibberellic acid on Nigella sativa L. Acta Bot. Croat.2007a; 66: 67-73.
30. Huttly AK, Phillips AL. Gibberellin-regulated plant genes. Physiol. Plant. 1995; 95: 310-317.
31. Buchanan BB, Gruissen W, Jones RL. Biochemistry and Molecular Biology of Plants. American Society of Plant physiologists, Rockville, 2000; Maryland.
32. Marschner, H. Mineral Nutrition of Higher Plants. 2nd Ed. Academic Press, 2002; London.
33. Estruch JJ, Pereto, JG, Vercher Y, Beltran JP. Sucrose loading in isolated veins of *Pisum sativum*: Regulation by Abscissic acid, gibberellic acid and cell turgor. Plant Physiol.1989; 91: 259-265.
34. Akter A, Ali E, Islam MMZ, Karim R, Razzaque AHM. Effect of GA_3 on growth and yield of mustard. Int. J. Sustain. Crop Prod. 2007; 2 (2): 16-20.
35. Khan, T.A, Mazid, M and Quddusi, S. Role of organic and inorganic chemicals in plant-stress mitigation. Approaches to Plant Stress and their Management (Ed.). P.K. Gaur and P. Sharma. DOI-10.1007/978-81-322-1620-9-3. Springer, India, 2014. Pp. 39-52.
36. Quddasi S, Khan F, Mazid M, Khan TA, Shakeel A, Arshad M. Quality status of nutrients, vitamins and hormones under pathological threats in tomato: Basic scenario. Medi. Chemis. Anal.2014; 4(1):12-21.
37. Naqvi N, Khan TA, Mazid M, Khan F, Quddasi S, Choudhury RR. Phytoremediatory potential of Guava and Ashok tree at three different sites of Bareilly district-A case study: ARPN Journal of Agricultural and Biological sciences. 2014; 9(3): 101-109.
38. Mozer TJ. Control of protein synthesis in barley aleurone layers by the plant hormones gibberellic acid and abscissic acid. Cell.1980; 20: 479-485.
39. Khafagy MA. Effects of growth substances, biological boron and their combination on soybean plants under nitrogen levels. J. Agric. Sci. Mansoura Univ. 1995; 20: 4641–4658.

Source of support: Nil, Conflict of interest: None Declared