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ENHANCING IMPACT OF GIBBERELLIC ACID ON GROWTH, PHOTOSYNTHETIC ENZYMES, PRODUCTIVITY AND TOTAL CARBOHYDRATE CONTENT OF CHICKPEA - A TRADITIONAL (ANTIDIABETIC) HERBAL PULSE

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ABSTRACT

India is the main chickpea (*Cicer arietinum* L.) producing country in Asia, despite there are few studies on gibberellic acid (GA) application to chickpea. Production of chickpea, as a forage and food source, has increasingly become a popular agricultural practice in India but cultivation of chickpea for both seed and forage is not keeping pace with increasing demand for protein based products. A pot experiment was conducted at a net house in the 'rabi' season of 2017 to 2018 to evaluate the effects of gibberellic acid (GA) on shoot dry weight, nitrate reductase activity (NR), carbonic anhydrase activity (CA), seed yield and seed protein and carbohydrate content. Sterilized seeds of chickpea were soaked in four different concentrations viz., 0, 10^{-7}, 10^{-6} and 10^{-5}M solution of GA for 4, 8, or 12 h and sown in pots. The potted plants were then analysed at 90 and 100 days after sowing (DAS) for shoot dry weight per plant, NR, CA activity. Seed yield, seed protein and carbohydrate content were estimated at harvest. All parameters were found to be significantly enhanced by the soaking with different levels of GA, with optimum stimulation being noted following an 8-h soaking treatment with 10^{-6}MGA. The total seed protein and carbohydrate content were stimulated by 82.69% and 11.00% respectively.

KEYWORDS

Dry weight, Carbonic anhydrase, Nitrate reductase activity, Carbohydrate content, Seed protein content, Gibberellic acid and Seed yield.

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INTRODUCTION

The pulses by virtue of having almost twice the amount of protein in comparison with cereals make a major contribution to human diet in developing countries of tropical and sub-tropical areas (Rochfort and Panozzo, 2007)[1]. This crop is grown on 8.21 million hectares of our country with the annual production of 7.48 million tonnes and average productivity of 911 kg/ha (FAO, 2012)[2]. Though chickpea is grown in our country in the largest area in comparison with the other countries of the world, but her productivity at 911kg/ha is much lower than those of the developed countries of world, such as 2833.3kg/ha of China, 1668.4 kg/ha of Canada and 1488.6kg/ha of USA (FAO, 2012)[2].

Among pulses, for production, chickpea occupies the first position in India and third position at global level (FAO, 2012)[2]. It is very nutritive and used as a protein adjunct to starchy diets. It is given as preventive diet to atherosclerosis 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 diseases, 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 (Anonymous, 1992)[3]. The production of chickpea has not keeping pace with the increasing domestic demands. As mentioned earlier, there is limitation on increasing the acreage for cultivation, it is, therefore, highly logical to innovate ways that can improve the productivity. To attain such goal, the use of GA may play an important role as this is known to affect many facets of plant life (Davies, 2004)[4]. GA occupies a prominent position in mediating a variety of plant physiological processes including seed germination, leaf expansion, flower and fruit set, dry matter production, photosynthesis, translocation of food material and synthesis of mRNA coding for hydrolytic enzymes (Shah and Samiullah, 2007[5], Tiwari *et al*, 2011)[6]. The superiority of GA to the among growth regulators has also been substantiated in the author's primitive studies also. GA is known to exert their effects with help of specific enzymes, the synthesis of which induce by influencing the translation and/or transcription (Huttly and Phillips, 1995)[7]. Hence, even though GA itself may be metabolized, its future positive and effective consequences remain apparent because of these enzymes. Therefore, the seed priming application of GA used before sowing may strongly alter the vegetative growth pattern during which the basic infra-structure of crop is established/ its physiology and metabolic pathways. Keeping its prominent role in various physiological processes of plants, it is logical to exploit its potential by way of establishing its adequate level and soaking duration for pre-sowing seed treatment. The aim of the experiment was to establish the most effective concentration and duration of pre-sowing seed treatment of GA for improving the performance of chickpea cultivar DCP 92-3.

MATERIAL AND METHODS

A pot experiment was conducted during the 'rabi' (winter) season on chickpea cultivar (DCP 92-3) in a net house of the Department of Botany, Aligarh Muslim University, Aligarh. Aligarh district has the same soil composition and the appearances as those found generally in the plains of western Uttar Pradesh (Northern India). It is situated at 27.88 ^{o}N latitude (www.timehall.com), 78.08^{o}E longitude and 180m average altitude with an area of 3700.4 sq km (www.maps of india.com). Its climate is sub-tropical, with severest hot dry summers and intense cold winters. The winter extends from the middle of October to the end of March. The average temperatures for December and January are about 15^{o}C and 13^{o}C respectively. The average rainfall is 847.3 mm. More than 85% of the total rainfall occurs during a short span of four months from June to September and the remaining showers are received during winter season, useful for rabi crops. The soil sample was analysed in the Soil Testing Laboratory, Government Agriculture Farm, Quarsi, Aligarh for various physico-chemical properties. Before sowing, the earthen pots of equal size (25cm height x 25cm diameter) were filled with the homogenous mixture of soil and FYM in the ratio of 4:1 at the rate of 5kg /pot. The physico-chemical analysis of the mixture of soil and FYM used for filling of the pots is given in Table No.1. The required number of pots was arranged according to a factorial randomized design. Just one day before the sowing, pots were irrigated lightly to provide necessary moisture for seed germination. Authentic seeds of the high yielding cultivar of chickpea, namely DCP 92-3 was obtained from the IIPR, Kanpur (Uttar Pradesh). After selecting seeds of uniform size, their viability was tested. The healthy seeds were soaked with double distilled water (DDW) for 2 h and then were surface sterilized with absolute ethyl alcohol followed by repeated washing with DDW. Subsequently, seeds were inoculated with the recommended strain of *Rhizobium* namely TAL 1148 and then were sown in earthen pots. Prior to the foliar treatments, 100milli-litre (Ml) stock solutions of GA (SIGMA USA) at 10^{-3}M were prepared. The amount of GA was dissolved in 10Ml ethyl alcohol and the final volume was made 100Ml using DDW. Further dilutions of the stock solutions were made with DDW as per requirement. Four concentrations of aqueous solution of GA for pre-sowing seed treatment, viz. 0 (water), 10^{-7}, 10^{-6} and 10^{-5}M GA, constituted one variant and the three pre-sowing seed soaking durations, i.e. 4, 8 and 12 hours (h), the other. A uniform recommended basal dose of 40kg N + 30kg P_2O_5/ha (17.9mg N + 13.4mg P/kg soil) was applied to all pots (Anonymous,1992)[3], 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 30 DAS. Diammonium phosphate was used as a common source for N and P. However, the remaining amount of N dose was compensated with urea. Finally, four plants per pot were maintained. A water-sprayed control was also included in the scheme of treatments. The experiment was performed according to a factorial randomized design. There were four replicates for each treatment. The summary of the experiment is given in Table No.2. The pots were kept free from weeds and irrigated as and when required for better establishment. Subsequent irrigation was done two times a week to keep an optimum moisture level in the soil.

The performance of the crop was assessed with regard to shoot dry weight, CA, NR at 90 and 100 DAS and seed yield and seed protein and carbohydrate content at harvest.

Sampling techniques

One plant from each replicate was uprooted randomly at the various sampling stages in all experiments to assess the performance of the crop on the basis of growth characters, physiological and biochemical characteristics, yield attributes and quality parameter. Growth characters and physiological and biochemical characteristics were studied at 90 and 100 DAS while yield and quality parameters at harvest.

Growth parameters

The shoot of each plant were dried in a hot air oven at 80^{o}C for 24 h and their dry weight was obtained separately with the help of an electronic balance.

Assays for photosynthetic enzymes

Carbonic anhydrase (CA) activity was determined in fresh leaves collected randomly from each replicate. The enzyme CA catalyzes the reversible hydration of CO_2 to give the bicarbonate ion (HCO_3^-). The activity of the enzyme was estimated by adopting the method of Dwivedi and Randhawa (1974)[8]. Finally, the activity of the enzyme was expressed in terms of mol CO_2kg^{-1} (leaf fresh mass) s^{-1}.

The enzyme, NR catalyses the reduction of $NO_3^-$to nitrite (NO_2^-). The NR activity in fresh leaves was estimated by the method of Jaworski (1971)[9].

Yield attributes

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.

The total seeds of two plants were threshed, cleaned and allowed to dry in the sun for some time and their weight was obtained with the help of an electronic balance, with expressing their weight on per plant basis. The seed protein and carbohydrate content in the dry seeds was estimated by adopting the methodology of Lowry *et al*, (1951)[10] and Dubois *et al*, (1956)[11].

Statistical analysis

All data were analysed statistically adopting the analysis of variance technique, according to Gomez and Gomez (1984)[12]. 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.

RESULTS

In this factorial randomized pot experiment, the effect of four pre-sowing seed soaking concentrations of GA and of three soaking durations, alone or in combination, was studied on the performance of chickpea cultivar DCP 92-3. The effect of the pre-sowing seed-soaking concentrations of GA and of the soaking durations, alone or in combination, was significant on all parameters studied at two sampling stages (90 and 100 DAS). The effect of the pre-sowing seed soaking concentrations of GA and the soaking durations, alone or in combination, was significant on all parameters studied at 90 and 100 DAS, except the interaction effect on NR activity at 100 DAS and seed yield at harvest.

Shoot dry weight per plant

Soaking treatment $S_{10^{-6}M\,GA}$ proved best at both stages. Its effect was followed by that of $S_{10^{-7}M\,GA}$ at each stage. Soaking with $S_{10^{-6}M\,GA}$ gave 30.77 and 31.85% higher value at 90 and 100 DAS respectively than SW. Soaking duration S_{8h} gave the maximum value at both stages. Its effect was followed by that of S_{4h} and S_{12h} at each stage. Soaking duration S8h gave 13.29 and 13.33% higher value at 90 and 100 DAS respectively than the lowest value giving soaking duration S_{12h}. Interaction $S_{10\text{-}6M\,GA} \times S_{8h}$ gave the maximum shoot dry weight per plant at both stages. Its effect was followed by that of $S_{10^{-7}M\,GA} \times S_{8h}$, $S_{10^{-6}M\,GA} \times S_{4h}$, and $S_{10^{-7}M\,GA} \times S_{4h}$ at each stage. Interaction $S_{10\text{-}6M\,GA} \times S_{8h}$ gave 58.87 and 66.38% higher shoot dry weight per plant at 90 and 100 DAS respectively than the lowest value giving interaction $S_W \times S_{4h}$.

Carbonic anhydrase activity

Soaking treatment $S_{10^{-6}MGA}$ gave the maximum value at both stages. Its effect was followed by that of $S_{10^{-7}MGA}$ at each stage. Soaking with $S_{10^{-6}MGA}$ gave 44.50 % and 51.36 % higher value at 90 and 100 DAS respectively than S_W. Soaking duration S8h proved best at 90 DAS. Its effect was, however, at par with that of S_{12h} at this stage. At 100 DAS, soaking duration S_{12h} gave the maximum value, however, its effect was at par with that of S_{8h}. Soaking duration, S_{8h} gave 7.89% higher value at 90 DAS and soaking duration S_{12h}, 5.31% higher value at 100 DAS than the lowest value giving soaking duration S_{4h}. Interaction $S_{10\text{-}6MGA} \times S_{8h}$ gave the maximum value at both stages. Its effect was followed by that of $S_{10^{-7}M\,GA} \times S_{8h}$ and $S_{10^{-6}M\,GA} \times S_{4h}$ at 90 DAS and was, however, equalled by that of $S_{10^{-6}M\,GA} \times S_{12h}$, $S_{10^{-6}M\,GA} \times S_{4h}$ and $S_{10^{-6}M\,GA} \times S_{12h}$ at 100 DAS. Interaction $S_{10^{-6}M\,GA} \times S_{8h}$ gave 87.06 and 76.76% higher value at 90 and 100 DAS respectively than the lowest value giving interaction $S_W \times S_{4h}$ (Table No.4[8]).

Seed yield per plant

Soaking treatment $S_{10^{-6}M\,GA}$ gave the maximum value for seed yield. However, its effect was at par with that of $S_{10^{-7}M\,GA}$. Soaking with $S_{10^{-6}MGA}$ gave 86.69% higher value than S_W Soaking duration S_{8h} gave the maximum value. However, its effect was at par with that of S_{4h}. Soaking duration S_{8h} gave 5.44% higher value than S_{12h} which gave the lowest value. The interaction effect on this parameter was not found significant.

Seed protein and carbohydrate content

Soaking treatment $S_{10^{-6}M\,GA}$ gave the maximum value for seed protein and carbohydrate content. Its effect was followed by that of $S_{10^{-7}M\,GA}$. Soaking with $S_{10\text{-}6\,M\,GA}$ gave 27.34% and 34.56% higher value than S_W respectively for protein and carbohydrate content. Soaking duration S_{12h} gave the maximum value. Its effect was at par with that of S_{8h}. Soaking duration S_{12h} gave 78.89% and 87% higher value than the lowest value giving soaking duration S_{4h} for protein and carbohydrate content respectively. Interaction $S_{10^{-6}M\,GA} \times S_{8h}$ gave the maximum value for both. Its effect was followed by that of $S_{10^{-6}M\,GA} \times S_{12h}$ and $S_{10\text{-}6M\,GA} \times S_{4h}$ for seed protein content and $S_{10^{-6}M\,GA} \times S_{12h}$ for seed carbohydrate content. Interaction $S_{10^{-6}MGA} \times S_{8h}$ increased the seed protein and carbohydrate content by 26.01% and 11% over $S_W \times S_{12h}$ and by 54.32% and 34.39% over the least value giving combination $S_{10^{-5}M\,GA} \times S_{8h}$ respectively.

DISCUSSION

The performance of the crop has been assessed in terms of shoot dry weight, NR, and CA activities and also seed yield as well as quality attributes viz., seed protein and carbohydrate content. The results have been discussed parameter-wise in the light of the knowledge of the subject and research work undertaken by other pulse crop scientists below. The enhancing effect of application of GA at 90 and 1000 DAS over the water-sprayed control on shoot dry weight per plant of chickpea cultivar, DCP 92-3 receiving the officially recommended basal dose of 40kg N + 30kg P2O5/ha can be traced to its various comparatively more roles in plants. For example, application of GA improves, among other processes, absorption and use efficiency of nutrients (Sandhya *et al*, 2012)[13], activity of enzymes (Chanda *et al*, 1998[14], Sandhya *et al*, 2012)[13], cell division and cell enlargement (Buchanan *et al*, 2000[15], Marschner, 2002[16], Taiz and Zeiger, 2010)[17], chlorophyll content (Afroz *et al*, 2005)[18], elongation of internode (Tiwari *et al*, 2011)[6], membrane permeability (Taiz and Zeiger, 2010)[17], PN (Afroz *et al*, 2005)[18], nucleic acid and protein synthesis (Tiwari *et al*, 2011)[6], and transport of photosynthates (Ouzounidou and Ilias, 2005)[19].

In view of its crucial roles in different facets of plant life and very small quantity involved (economic), it is reasonable to rope in these above mentioned PGRs in innovative farm cultural practices. 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. As the C fixing ability of plants is influenced by mineral elements among other factors, the availability of P and S to leguminous plants affects production of dry matter and partitioning of photosynthates (Kharche *et al*, 2006[20], Chaurasia and Chaurasia, 2008)[21].

Further, it is gratifying to note that these data have been confirmed beyond doubt the superiority of application of GA over water-sprayed control. These results broadly corroborate the findings of earlier workers including Iqbal *et al*, (2001)[22], Yadav and Bharud (2006)[23], Mobin *et al*, (2007)[24]. The growth improving effect of pre-sowing seed treatment for 8 h with 10^{-6}M GA over their respective water treated control on NR and CA activities studied at 90 and 100 DAS of DCP 92-3 cultivar of chickpea grown with the recommended basal dose of N and P could be explained on the basis of its roles mentioned earlier and the fact that the supply of GA by pre-sowing seed treatment would more than compensate the 'hidden hunger' of growing crops for GA. Similar results have been obtained by a few workers including, Shah (2007 a, b)[25,26], Jafri (2009)[27] and Thakare *et al*, (2011)[28].

Improvement in shoot dry weight per plant of chickpea cultivar DCP 92-3 would has 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 emphasizing a significant and positive contribution towards these growth parameters.

The augmenting effect of seed soaking GA over the water-sprayed control on CA and NR activities of chickpea cultivars particularly DCP 92-3, receiving the recommended basal dose of 40 kg N + 30 kg P_2O_5/ha, studied at 90 and 100 DAS is worth mentioning. The increase in CA and NR activities can be attributes to the hormone-induced increase in transcription and/or translation of the gene that codes for CA (Sugiharto *et al*, 1992)[29] and NR (Roth-Benjerano and Lips, 1970)[30] to its role in enhancing the permeability of membranes and absorption of nutrients (Hopkins and Huner, 2009[31], Taiz and Zeiger, 2010)[17]. These results are also in accordance with the data of earlier workers including, Shah (2007a)[25] on CA activity; Sekhon *et al*, (1991)[32], Premabatidevi (1998)[33], Afroz *et al*, (2005)[18], Shah (2008)[34], Mazid and Khan (2017 a and b)[35,36] on NR activity.

The enhancing effect of pre-sowing seed treatment for 8 h with 10-6M GA over their respective water treated control on CA and NR activities of DCP 92-3 cultivar of chickpea grown with the recommended basal dose of N and is a noteworthy observation. This may also be attributed, as for growth characters, to its (GA) roles on one hand and compensation of the 'hidden hunger' for GA by its pre-sowing seed treatment on the other. These results also corroborate the findings of Shah (2007 a, b)[25,26] and Jafri (2009)[27] on CA activity, of Shah (2007a)[25] and Jafri (2009)[27] on NR activity and of Jafri (2009)[27], Mazid and Khan (2015)[37], Mazid and Jafri (2015)[38] for pre-sowing seed treatment.

Enhanced rate of CA activity of chickpea cultivar DCP-92-3 would have resulted in improving the PN and gs of treated plants. Likewise, increased NR activity might be responsible for increasing biosynthesis of chlorophylls that in turn would have improved PN of treated plants. Higher levels of leg haemoglobin content would also be responsible for increased content of chlorophylls leading to higher PN. This proposition is further confirmed by correlation studies emphasizing a positive and significant correlation between these pairs of parameters (Mazid *et al*, 2017)[39].

The increase in the number of pods per plant and 100-seed weight resulting from the foliar application of GA in comparison with the water-sprayed control (studied at harvest) of chickpea cultivar DCP 92-3 receiving the recommended basal dose of N and P is worth mentioning. The increase in the above yield attributes may be traced to its various roles leading to observed higher values for shoot dry weight per plant and, NR and CA activities of treated plants. Moreover, it mediates differentiation (Huttly and Phillips, 1995[7], Mobin, 1999[40], Afroz *et al*, 2005[18], Mazid and Naqvi, 2014 a and b)[41,42] leading to enhanced number of flowers which develop into pods. As mentioned earlier it plays role in cell division and cell enlargement (Liu and Loy, 1976[43], Moore, 1989[44], Huttly and Phillips, 1995[7], Arteca, 1996[45], Buchanan *et al*, 2000[15], Marschner, 2002[16], Taiz and Zeiger, 2010)[17] resulting in proper development of under-developed pods especially at the terminal end of branches; PN (Afroz *et al*, 2005)[18] supplying sufficient C skeleton; and membrane permeability (Wood and Paleg, 1972[46], Crozier and Turnbull, 1984[47]) and transport of photosynthates (Mulligan and Patrick, 1979[48], Aloni *et al*, 1986[49], Daie *et al*, 1986[50], Estruch *et al*, 1989[51] and Khan, 2008)[52] favouring partitioning hence higher values for the yield parameters of treated plants. These results broadly corroborate the findings of Yadav and Bharud (2006)[23], Akter *et al*, (2007)[53], Tripathi *et al*, (2007)[54], and Shah and Samiullah (2007)[5], Mazid (2014)[55].

The augmenting effect of pre-sowing seed treatment with 10-6M GA for 8 h over water-soaking treatment on pods per plant and seeds per pod and of spray treatment at 60 and 70 DAS on chickpea cultivar DCP 92-3 grown with a recommended basal dose of N and P, is understandable. This may be due to its roles mentioned earlier for improving these parameters in and offset of the 'hidden hunger' for GA by its pre-sowing seed treatment or foliar application. Similar results were also obtained by Arif (2002)[56], Mazid and Roychowdhary (2014)[57], Khan and Samiullah (2003), Shah (2007a)[25] and Jafri (2009)[27] on pre-sowing seed treatment of GA.

The increased yield attributing parameters of treated plants, particularly pods per plant and 100-seed weight are likely to have contributed to the improved seed yield. 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 and carbohydrate content due to pre-sowing seed treatment of GA is not surprising. An improvement in protein synthesis may result from the application of GA (Mozer, 1980)[58], hence higher values for seed protein content. These results broadly corroborate with the findings of Khafagy (1995)[59] on GA application and of Jain *et al*, (1999)[60], Kumar *et al*, (2003)[61], Kharche *et al*, (2006)[20], Mazid *et al*, (2014)[62], Mazid and Khan (2015 a and b)[63,64], Mazid and Naz (2017 a and b)[65,66] Jafri *et al*, (2015)[67], Hassanpourgdham *et al*, (2015)[68], Naqvi *et al*, (2014)[69] and Mansur *et al*, (2009)[70] on P and S although on basal application. The best concentration (10^{-6}M) and duration (8_h) of pre-sowing seed treatment of the selected PGR (GA) have been established for the optimum performance of the most promising cultivar of chickpea (DCP 92-3).

Table No.1: Physico-chemical characteristics of the mixture of soil and farmyard manure used for experiment

S.No	Soil characteristics	Experiment (2011-2012)
	Texture	**Sandy loam**
2	pH (1:2)	7.87
3	E.C. (dSm^{-1})	0.67
4	Available N (kg N /ha)	188.00
5	Available P (kg P_2O_5 /ha)	28.40
6	Available K (kg K_2O/ha)	224.00
7	Calcium carbonate (%)	0.06

Table No.2: Summary of Experiment 2 (2009-2010)

S.No	Soaking durations (S_h)	Soaking concentrations (S_{MGA})			
		S_W	$S_{10^{-7}MGA}$	$S_{10^{-6}MGA}$	$S_{10^{-5}MGA}$
1	S_{4h}	-	-	-	-
2	S_{8h}	-	-	-	-
3	S_{12h}	-	-	-	-

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

Concentrations (C) : 4
Durations (D) : 3
Replicates : 4
Interactions (CxD) : 12
Design : Factorial randomized

Table No.3: Effect of concentrations (C) and durations of pre-sowing seed treatment (D) of GA on shoot dry weight per plant (g) of chickpea cultivar DCP 92-3 at two growth stages (mean of four replicates)

S.No	Soaking durations (S_h)	Soaking concentrations ($S_{M\,GA}$)				Mean
		S_W	$S_{10^{-7}M\,GA}$	$S_{10^{-6}M\,GA}$	$S_{10^{-5}M\,GA}$	
90 DAS						
1	S_{4h}	1.24	1.57	1.65	1.42	1.47
2	S_{8h}	1.50	1.67	1.99	1.51	1.62
3	S_{12h}	1.34	1.50	1.47	1.40	1.42
4	Mean	1.30	1.58	1.70	1.44	
5	C.D. at 5%		C = 0.059	D = 0.068	C x D = 0.114	
100 DAS						
6	S_{4h}	1.19	1.67	1.77	1.59	1.56
7	S_{8h}	1.49	1.72	1.99	1.61	1.70
8	S_{12h}	1.38	1.52	1.60	1.48	1.50
9	Mean	1.35	1.64	1.78	1.56	
10	C.D. at 5%		C = 0.062	D = 0.071	C x D = 0.120	

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

Table No.4: Effect of concentrations (C) and durations of pre-sowing seed treatment (D) of GA on carbonic anhydrase activity [molCO$_2$ kg^{-1}(F.M)s^{-1}] of chickpea cultivar DCP 92-3 at two growth stages (mean of four replicates)

S.No	Soaking durations (S_h)	Soaking concentrations ($S_{M\ GA}$)				Mean
		S_W	$S_{10^{-7}M\ GA}$	$S_{10^{-6}M\ GA}$	$S_{10^{-5}M\ GA}$	
90 DAS						
1	S_{4h}	2.134	3.410	3.720	3.205	3.117
2	S_{8h}	2.371	3.740	3.992	3.347	3.363
3	S_{12h}	2.274	3.330	3.529	3.200	3.333
4	Mean	2.593	3.493	3.747	3.251	
5	C.D. at 5%		C = 0.128	D = 0.148	C x D = 0.250	
100 DAS						
6	S_{4h}	2.660	4,010	4.505	3.920	3.774
7	S_{8h}	3.112	4.100	4.702	3.747	3.915
8	S_{12h}	3.364	4.205	4.621	3.724	3.979
9	Mean	3.045	4.105	4.609	3.797	
10	C.D. at 5%		C = 0.152	D = 0.175	C x D = 0.296	

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

Table No.5: Effect of concentrations (C) and durations of pre-sowing seed treatment (D) of GA on nitrate reductase activity (n mol NO_2^-/g/ (leaf F W)/h) of chickpea cultivar DCP 92-3 at two growth stages (mean of four replicates)

S.No	Soaking durations (S_h)	Soaking concentrations ($S_{M\ GA}$)				Mean
		S_W	$S_{10^{-7}M\ GA}$	$S_{10^{-6}M\ GA}$	$S_{10^{-5}M\ GA}$	
90 DAS						
1	S_{4h}	287.72	329.37	367.14	319.72	325.99
2	S_{8h}	302.14	349.30	405.41	302.52	339.84
3	S_{12h}	300.19	319.45	352.17	300.12	317.98
4	Mean	296.68	332.71	374.91	307.45	
5	C.D. at 5%		C = 12.676	D = 14.635	C x D = 24.704	
100 DAS						
6	S_{4h}	305.42	392.82	407.47	337.24	360.74
7	S_{8h}	327.81	400.24	411.12	387.13	381.62
8	S_{12h}	319.45	374.58	401.49	330.18	356.43
9	Mean	317.56	389.21	406.69	351.58	
10	C.D. at 5%		C = 14.187	D =16.382	C x D = NS	

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

Table No.6: Effect of concentrations (C) and durations of pre-sowing seed treatment (D) of GA on seed yield per plant (g) of cultivar DCP 92-3 of chickpea at harvest (mean of four replicates)

S.No	Soaking durations (S_h)	Soaking concentrations ($S_{M\ GA}$)				Mean
		S_W	$S_{10^{-7}M\ GA}$	$S_{10^{-6}M\ GA}$	$S_{10^{-5}M\ GA}$	
1	S_{4h}	2.51	4.18	4.34	4.10	3.74
2	S_{8h}	2.52	4.27	4.47	4.21	3.87
3	S_{12h}	2.57	4.21	4.24	4.11	3.67
4	Mean	2.33	4.22	4.35	4.14	
5	C.D. at 5%		C = 0.150	D = 0.173	C x D = NS	

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

Table No.7: Effect of concentrations (C) and durations of pre-sowing seed treatment (D) of GA on seed carbohydrate content (%) of cultivar DCP 92-3 of chickpea at harvest (mean of four replicates)

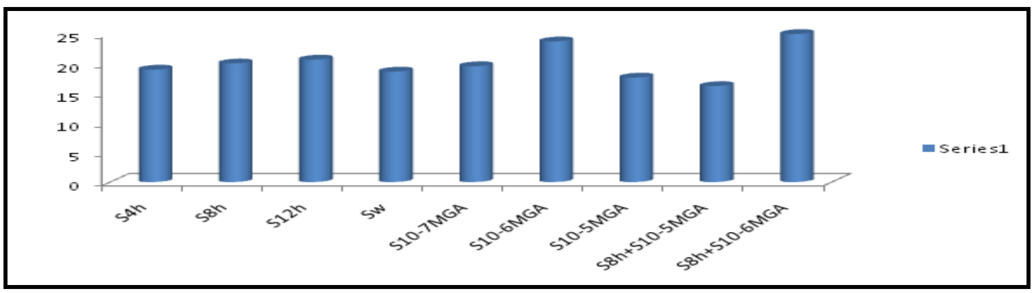
S.No	Soaking durations (S_h)	Soaking concentrations ($S_{M\ GA}$)				Mean
		S_W	$S_{10^{-7}M\ GA}$	$S_{10^{-6}M\ GA}$	$S_{10^{-5}M\ GA}$	
1	S_{4h}	45.90	45.86	50.78	40.95	45.87
2	S_{8h}	44.00	50.34	53.85	42.09	47.28
3	S_{12h}	39.60	43.95	49.35	46.90	44.95
4	Mean	43.17	46.72	51.32	43.32	
5	C.D. at 5%		C = 1.08	D = 1.20	C x D = 1.32	

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

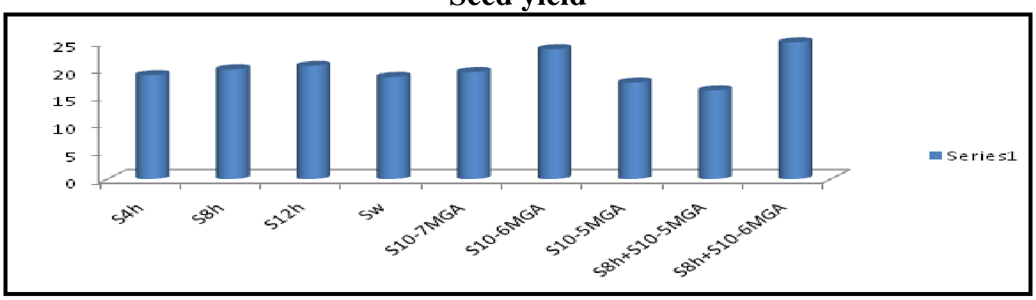
Table No.8: Effect of concentrations (C) and durations of pre-sowing seed treatment (D) of GA on seed protein content (%) of cultivar DCP 92-3 of chickpea at harvest (mean of four replicates)

S.No	Soaking durations (S_h)	Soaking concentrations ($S_{M\ GA}$)				Mean
		S_W	$S_{10^{-7}M\ GA}$	$S_{10^{-6}M\ GA}$	$S_{10^{-5}M\ GA}$	
1	S_{4h}	17.20	18.47	22.80	17.50	18.99
2	S_{8h}	18.90	20.00	25.00	16.20	20.03
3	S_{12h}	19.84	20.20	23.45	19.23	20.68
4	Mean	18.65	19.56	23.75	17.64	
5	C.D. at 5%		C = 0.767	D = 0.886	C x D = 1.495	

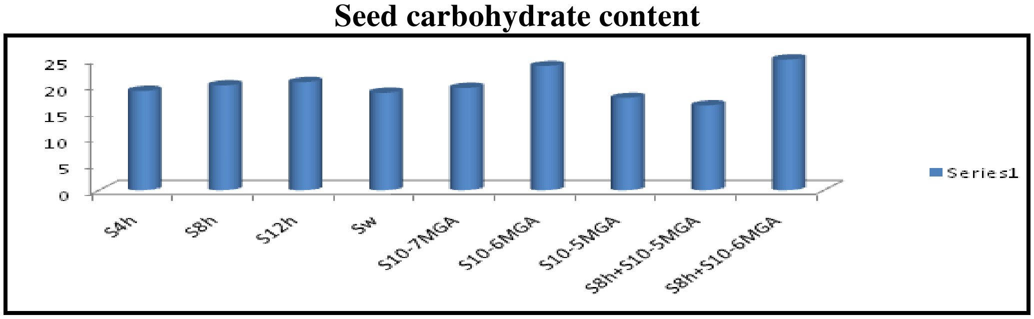
N.B.: A uniform basal dose of 40kg N + 30kg P_2O_5 /ha was applied to all plants.



Seed yield



Seed carbohydrate content



Seed protein content

CONCLUSION

The enhancing effect of pre-sowing seed treatment for 8 h over their respective water treated control on CA and NR activities and nutrient content of DCP-92-3 cultivar of chickpea grown with the recommended basal dose of N and P is a noteworthy observation. This may also be attributed, as for growth characters, to its (GA) roles on one hand and compensation of the hidden hunger for GA by its pre-sowing seed treatment or foliar application on the other.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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