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Original Article

Impact of Seed Priming Application of Gibberellic Acid on Development, Harvesting Attributes and Total Energy Value of Bengal Gram

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ABSTRACT

The chickpea also known as Gram or Bengal gram, and it is the most significant and well recognized pulse of India; India, largest producer, contributing highest share in area as well as production. It is widely referred as an excellent beam of protein, especially in developing Nations and it is relatively of minor significance in global market but it is extremely good for local trade in numerous tropical and subtropical areas. A pot research experiment conducted during the *'rabi'* season of 2023-2024 on chickpea cultivar (PUSA-334) in a net house of the Department of Botany (Agronomy), SVU, Amroha to access the effects of gibberellic acid (GA) on shoot dry weight (SDW), nitrate reductase activity (NRA), carbonic anhydrase activity (CAA) activity seed yield (SY) and seed protein as well as carbohydrate content. Sterilized seeds of variety, PUSA 334, soaked in four different concentrations viz., 0, 10^{-4}, 10^{-6} and 10^{-8} M solution of GA for 6, 8, or 10 h and sown in these pots. The potted plants then analysed at 100 and 110 days after sowing (DAS) for SDW/plant, NRA, & CAA. In addition, SY, seed protein and carbohydrate content estimated at harvesting time (120 DAS). All parameters reported significant and promoted by the soaking with different levels of GA, however, with highest stimulation reported for a 10-h soaking treatment (duration) with 10^{-6} MGA. The total seed protein and total carbohydrate content stimulated much greatly by this approach and results are better than still any other approach applied for chickpea growth and quality values enhancement.

KEYWORDS

• Shoot dry weight • Carbonic anhydrase activity • Nitrate reductase activity • Protein content • Gibberellic acid • Seed yield • carbohydrate content

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INTRODUCTION

Being rich and cheap source of protein, it helps the people to improve the nutritional quality of the diet. Although, chickpea is relatively minor importance in global market. The pulses having almost triple amount of protein as compare to cereals crops (Rochfort and Panozzo, 2007). Mostly chickpea is grown in South Asia, which accounts for more than 75% of the global chickpea area (Reddy *et al.* 2013). Chickpeas account for almost half of production of pulses in India. Major states in India that produce this wonderful pulse include Maharashtra (25.97% Nation's production), Madhya Pradesh (18%), Rajasthan (20%), Gujarat (10%), and U.P. (5%). In local market, chickpea consumption is varied (DES, 2023). It occupies the first position in India and third position globally (DES, 2023). However, its production has not keeping pace with the increasing domestic demands. India produced 13.75 million tonnes of chickpeas in 2021–2022, with the productivity of 12.6 q/ha, on 10.91 million ha of land (DES 2023).There is limitation on increasing the acreage for cultivation, so it is only logical to create and develop strategies, can improve the productivity of this crop in India (Ray *et al*., 2013). The total area under pulse in India increased from 19 million hectares in 1950–51 to 28.8 million hectares in 2021–22 (Anonymous, 2022).

The majority of Indian farmers hardly use fertilize or use FYM when growing pulses in their field. Global crop productivity needs to full double by 2050 (Ray *et al.,* 2013) to meet the growing population demand (Vollset *et al*., 2020). Udayana *et al.* (2021) pointed out that managing water and nutrients is essential to achieving expected production demands. The average yield and yield potential differ significantly, thus there is a chance to increase productivity by optimal management and fertilization practices.

To attain such goal, the use of GA may play an important role because it is to affect many physiological aspects facets of plant life (Tiwari *et al*., 2011). Among the various barriers attributing to low productivity include poor soil fertility, traditional method of growing and non-availability of improved techniques. The superiority of GA_3 among other phytohormones substantiated in the different primitive studies already (Jafri et. al, 2009; Mazid *et al*., 2014). They reported to impose their effects through the help of some significant and specific enzymes that stimulate through influencing the translation and/or transcription factors (Kumar *et al*., 2024). Hence, even though GA itself may be metabolized, its future positive and effective consequences remain apparent because of these biocatalysts.

Therefore, the seed priming roles of GA used before sowing may strongly influence the vegetative growth system during which the basic infra-structural plan established as well as its biochemistry, physiology and anabolic-catabolic pathways also improved. Keeping its much significant role in numerous physio-biochemical processes, it is logical to exploit its potential by way of establishing its optimum usable concentration (amount) and soaking duration/hours for pre-sowing seed treatment.

The aim of experiment is to establish an effective concentration (usable) and tolerable duration of pre-sowing treatment (time period/hours) of this wonderful PGR for improving the performance of chickpea cultivar, PUSA 334.

MATERIALS AND METHODS

Before sowing, the various fully complete earthen pots of equal size (25 cm height x 20 cm diameter) filled by mixture of soil and FYM in ratio of 4:1 at the rate of 5 kg in each pot. The chemical analysis of the mixture and FYM used reported as; texture, sandy loam; pH, 8; E.C, 0.65; N-P-K (Kg/ha), 210-34.32-209.20 respectively and calcium carbonate (%) reported as 0.15.

A research experiment conducted during the '*rabi*' season of 2023-2024 on cultivar (PUSA-334) in a net house of the Department of Botany (Agronomy), SVU, Amroha. Amroha district has the same soil composition and the appearances as those found in the plains of central-western part of the state, U.P. It is situated at 28.23 ˚N latitude, 72.01˚E longitude and 190 m average altitude with an area of 3800.4 sq km. Its climate is sub-tropical to some extent, with hot dry summer season and more cold winter season exist. The temperatures for December and January are 18˚C and 134˚C respectively. The average rainfall is 837.6 mm. More than 75% of the total rainfall occurs during a time period of 4 months from June to September and somewhat November and the remaining showers received during winter season rarely. The good and vibrant quality seeds of the varieties of the Bengal gram like PUSA-334 purchased from the Indian Institute of Pulse research (IIPR) Kalyanpur, Kanpur. After selecting the seeds of uniform size, healthy, and later on their viability tested through a short experiment before planting this experiment. Seeds inoculated with *Rhizobium* (JKLM 19E) and then sown in already agronomically prepared pots. 100 milli-litre (Ml) stock solutions of GA at 10^{-3}M prepared in advance for further application to pots in experimentation at sufficient time intervals as we needed. The amount of GA dissolved in 10 Ml ethyl alcohol and last volume prepare up to 100 ML by applying the amount of DDW at suitable rate

A uniform suggested recommended basal dose of 35 kg N + 30 kg P_2O_5/ha (15.3 mg N+14.2 mg P/kg soil) applied uniformally to pots with the half dose of N and complete dose of P at the time of sowing and the remaining half dose giving N after 50 DAS. DAP used as a common source for N and P to a greater or somewhat extent as other fertilizer used. However, the remaining amount of N dose adjusted with main field organic fertilizers, called urea. Finally, four plantlets (replicates) per pot transferred. In addition, a water-sprayed control also included in the scheme of treatments of the experiment. The experiment carried out as per simple randomized design of experimental plan.

Sampling Techniques

One plant from each pot (replicate) taken randomly at the various sampling stages to measurement the performance on the basis of SDW/plant, CAA, NRA at 100 and 110 DAS and SDW/plant, and total seed protein and carbohydrate content at harvest. The shoot of each plant dried in a hot air oven at 80˚C for 24 h and their dry weight was obtained separately with the help of an electronic balance.

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 release the bicarbonate ion (HCO_3^-)

$$H_2O + CO_2 \xrightleftharpoons{CA} H^+ + HCO_3^-$$

The activity of the enzyme estimated by Dwivedi and Randhawa method (1974). The collected leaves cut in the small pieces (1cm^2) at temperature below 25˚ celsius (C). After mixing, 200 mg leaf pieces weighed, cut further into smaller size pieces keeping them in 100 ml 0.2 M aqueous cysteine hydrochloride solution in a Petridish at 0 to 5˚C for 25 minutes (min). The solution adhering on their surface thin removed with the help of a blotting paper. This followed by transfer immediately into a test tube having 4 ml phosphate buffer of pH 6.8. To this, 4 ml 0.2 M sodium bicarbonate in 0.2 M sodium hydroxide (NaOH) solution and 0.2 ml 0.002% bromothymol blue indicator added. After shaking, the tubes kept at 0-4˚C for 20 min. CO_2 liberated during catalytic action of the enzyme on sodium bicarbonate estimated by titrating the reaction mixture against 0.05 N hydrochloric acid (HCl) using methyl red as indicator. A control reaction mixture titrated against 0.05 N HCl. The difference of the sample reading and blank reading noted for further calculations of enzyme activity. The activity of the enzyme calculated by the following formula:

$$\frac{0.5 \times V \times N}{W \times T} \text{ m mol (CO2)mg}^{-1} \text{ (leaf fresh mass)min}^{-1}$$

Where,

V = Difference in volume (ml) of HCl used in blank and test sample titration

N = Normality of HCl

W = Fresh weight of tissue in mg

T = Duration of the catalytic action of the enzyme (min)

Finally, the activity of the enzyme expressed in terms of mol CO_2 kg^{-1} (leaf fresh mass)s^{-1}.

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 estimated by Jaworski approach (1971). The leaves cut into small pieces (1cm^2). Two hundred mg of these chopped leaves 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 added followed by the addition of 2.5 ml 5% isopropanol. These vials incubated in a BOD incubator for 2 h at 30±2˚C in the dark. 0.4 ml of incubated mixture taken into test tube to which 0.3 ml each of sulphanilamide and nephthyl-ethylenediamine-dihydrochloride solutions added. The test tube left for 20 min.

for maximum colure development. The mixture diluted to 5 ml using DDW. The OD recorded at 540 nm using the spectrophotometer.

SY /plant and yield components or variables determined from the other plants that not used for biomass at harvest at time of maturity of the crop varies as per area in case of India viz., Central Indian and Northern Indian. The total pods/seeds of two plants and number of seeds/pod weighted. The seed protein content in the dry seeds estimated by method of Lowry *et al.* (1951). The total protein content in the dry seeds estimated by adopting the methodology of Lowry *et al*. (1951). 50 mg oven dried seed powder taken into a centrifuge tube and 5 ml 5% trichloroacetic acid added. The solution shaken fully and allowed to stand for 30 min at room temperature. The solution centrifuged for 10 min at 4000 rpm and the supernatant discarded. To the residue, 5ml 1N NaOH added and mixed well. After 30 min, the solution centrifuged and the supernatant collected into a 50 ml volumetric flask. The residue washed twice with 5 ml 1N NaOH and the washings collected in the flask containing the supernatant. The volume of the flask made up to the mark with 1N NaOH. 1ml of this NaOH extract transferred to a 10 ml test tube and 5 ml of a reagent A (0.5% copper sulphate in 1% sodium tartarate in 1.1) added to it. The solution mixed well and allowed to stand for 10 min at room temperature. Another reagent B (carbonate-copper sulphate solution) at 0.5 ml added rapidly with immediate mixing. After waiting for 30 min, the solution turned blue. The intensity of the blue coloured solution measured with the spectrophotometer at 660 nm. A blank run with each sample.

Standard curve

50 mg egg albumin dissolved in 100 ml 1N NaOH in a 100 ml volumetric flask by heating the content on a heating plate. Ten different concentrations, i.e. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml from this solution taken into different test tubes and the volume reached to 1 ml. Into each test tube, 5 ml of a reagent 5% trichloroacetic acid (TCA), mixed well and allowed to stand for 10 min at room temperature followed by the addition of 0.5 ml reagent 1N NaOH rapidly with immediate mixing. After waiting for 30 min, the solution turned blue. The intensity of the colour read at 660 nm using the spectrophotometer. A blank used with each determination. Standard curve plotted using different concentrations of the working standard versus OD. With the help of this standard curve, the amount of protein present in the sample measured. The protein content noticed as the % as per dry weight basis.

Data Analysis

The data analyzed through analysis of variance technique (Gomez and Gomez, 1984). Replicates determined by F test and 'F' value found to be significant at 5% level of probability, CD (critical difference) noticed.

Sampling techniques

One plant from each replicate uprooted at random on the various sampling phases to assess the performance of the experiment on the basis of growth character (SDW), biochemical attributes (CAA, NRA), yield parameter (SY) and quality attributes of crop (protein and carbohydrate content). Growth characters and physiological and biochemical characteristics studied at 100 and 110 DAS while yield and quality parameters at harvesting time (120 DAS).

Growth parameters

The shoot of each plant dried in a hot air oven at 75°C for 72 h and their dry weight obtained separately through an electronic balance. The harvested plants sun-dried to prevent loss to a greater extent. After drying, each sample threshed individually. The seeds utilized assessing the other characteristics like SY/plant calculated by formula. The seed protein and carbohydrate content in the dry seeds estimated by adopting the methodology of Lowry *et al*. (1951) and Dubois *et al*. (1956).

RESULTS

In factorial randomized experiment, the effect of 4 pre-sowing seed soaking concentrations of GA (0, 10^{-4}, 10^{-6} and 10^{-8} M GA) and of 3 soaking durations (6h, 8h and 10h), alone or in combination, studied on performance of gram cultivar PUSA-334. The results (*Tables 3-8*) describe below.

Table 1: Physico-chemical features of the mixture of soil and (FYM) farmyard manure

Soil attributes	Experiment Time Period (2022-2023)
Texture	Sandy loam
pH (1:2)	7.87
E.C. (dSm^{-1})	0.64
Available N (kg N /ha)	185.00
Available P (kg P_2O_5 /ha)	27.40
Available K (kg K_2O/ha)	223.00
Calcium carbonate (%)	0.07

Table 2: Detail view of Experiment 2 (2022-2023)

Soaking durations (S_h)	Soaking concentrations (S_{MGA})			
	S_W	$S_{10^{-4}MGA}$	$S_{10^{-6}MGA}$	$S_{10^{-8}MGA}$
S_{6h}				
S_{8h}				
S_{10h}				

N.B.: A equal basal amount of 45 kg N + 35 kg P_2O_5/ha applied in pots.

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

Table 3: Impact of concentrations (C) and durations of pre-sowing seed treatment (D) of GA on shoot dry weight per plant (g) of chickpea cultivar PUSA 334 at two growth stages (100 and 110 DAS) (mean of four replicates)

Soaking durations (S_h)	Soaking concentrations (S_{MGA})				Mean
	S_W	$S_{10^{-4}MGA}$	$S_{10^{-6}MGA}$	$S_{10^{-8}MGA}$	
		100 DAS			
S_{6h}	1.20	1.54	1.62	1.40	1.47
S_{8h}	1.54	1.69	1.98	1.53	1.62
S_{10h}	1.51	1.51	1.49	1.41	1.42
Mean	1.31	1.54	1.74	1.43	
C.D. at 5%		C = 0.058	D = 0.069	C x D = 0.117	
		110 DAS			
S_{6h}	1.21	1.66	1.78	1.58	1.57
S_{8h}	1.71	1.71	1.98	1.60	1.71
S_{10h}	1.33	1.51	1.62	1.49	1.53
Mean	1.34	1.63	1.77	1.57	
C.D. at 5%		C = 0.063	D = 0.072	C x D = 0.124	

N.B.: A similar common basal dose of 45 kg N + 35 kg P_2O_5 /ha was applied to all pots.

N.B.: A similar common basal dose of 45 kg N + 35 kg P_2O_5/ha was applied to all pots.

Growth characters

The effect of the pre-sowing seed-soaking concentrations of GA and of soaking durations, alone or in combination, reported significant on concerned parameters studied at these sampling stages (100 and 110 DAS). In this experiment, the effect of 4 pre-sowing seed soaking concentrations and of 3 soaking durations, alone or in combination, studied on the performance of PUSA 334. The effect of the pre-sowing seed-soaking concentrations of GA and of the soaking durations, alone or in combination, significant on all parameters studied at two sampling stages (100 and 110 DAS). The effect of the pre-sowing seed soaking concentrations of GA and the soaking durations, alone or in combination is significant on all attributes studied at these 100 and 110 DAS except interaction impact on NRA at 110 DAS and SY at harvest.

Shoot dry weight per plant

Soaking treatment $S_{10^{-6}M\ GA}$ proved best at all these stages. Its effect followed by that of $S_{10^{-8}M\ GA}$ at each stage. Soaking with $S_{10^{-6}M\ GA}$ gave 32.72 and 33.82% higher value at 100 and 110 DAS respectively than S_W. Soaking duration S_{8h} provide highest value at both phases. Its effect followed by of S_{4h} and S_{10h} at each stage. Soaking duration S_{8h} gave 12.25 and 14.12% higher value at 100 and 110 DAS respectively than S_{10h}. Interaction $S_{10\text{-}6M\ GA} \times S_{8h}$ gave the highest shoot dry weight/plant at all stages. Its effect followed by $S_{10^{-8}M\ GA} \times S_{8h}$, $S_{10^{-6}M\ GA} \times S_{4h}$, and $S_{10^{-7}M\ GA} \times S_{4h}$ at each stage. Interaction $S_{10\text{-}6M\ GA} \times S_{8h}$ gave 56.81 and 64.31% more shoot dry weight/plant at 100 and 110 DAS respectively than $S_W \times S_{4h}$ (*Table* 4).

Table 4. Impact 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 PUSA 334 at two growth stages (100 and 110 DAS) (mean of four replicates)

Soaking durations (S_h)	Soaking concentrations (S_{MGA})				Mean
	S_W	$S_{10^{-4}MGA}$	$S_{10^{-6}MGA}$	$S_{10^{-8}MGA}$	
		100 DAS			
S_{6h}	2.132	3.412	3.721	3.206	3.118
S_{8h}	2.365	3.742	3.993	3.346	3.364
S_{10h}	2.270	3.334	3.529	3.202	3.334
Mean	2.590	3.492	3.746	3.253	
C.D. at 5%		C = 0.129	D = 0.147	C x D = 0.251	
		110 DAS			

Soaking durations (S_h)	Soaking concentrations (S_{MGA})				Mean
	S_W	$S_{10^{-4}\,MGA}$	$S_{10^{-6}\,MGA}$	$S_{10^{-8}\,MGA}$	
S_{6h}	2.662	4,011	4.506	3.922	3.773
S_{8h}	3.113	4.102	4.704	3.744	3.914
S_{10h}	3.362	4.206	4.623	3.723	3.978
Mean	3.044	4.107	4.608	3.795	
C.D. at 5%		C = 0.153	D = 0.174	C x D = 0.295	

Carbonic anhydrase activity

Soaking treatment $S_{10^{-6}\,MGA}$ gave highest value at all phases. Its effect followed by $S_{10^{-8}\,MGA}$ at each stage. Soaking with $S_{10^{-6}\,MGA}$ gave 43.20% and 53.16% higher value at 100 and 110 DAS respectively than S_W. Soaking duration S_{8h} proved better at 100 DAS. Its effect at par with S_{12h} at the stage. At 110 DAS, soaking duration S_{12h} provide the highest value, however, its effect at par with S_{8h}. S_{8h} gave 6.15% higher value on 100 DAS and soaking duration S_{12h}, 6.11% more at 110 DAS than S_{4h}. $S_{10\text{-}6MGA}$ × S_{8h} gave the higher at all stages. Its effect followed by $S_{10^{-7}\,M\,GA}$ × S_{8h} and $S_{10^{-6}\,M\,GA}$ × S_{4h} at 100 DAS and equalled by $S_{10^{-6}\,M\,GA}$ × S_{12h}, $S_{10^{-6}\,M\,GA}$ × S_{4h} and $S_{10^{-6}\,M\,GA}$ × S_{12h} at 110 DAS. $S_{10^{-6}\,M\,GA}$ × S_{8h} provide 85.02 and 74.13% greater at 100 and 110 DAS respectively than the lowest value providing interaction effect of S_W × S_{4h} (*Table* 5).

Table 5: Impact 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 PUSA 334 at two growth stages (100 and 110 DAS) (mean of four replicates)

Soaking durations (S_h)	Soaking concentrations (S_{MGA})				Mean
	S_W	$S_{10^{-4}\,MGA}$	$S_{10^{-6}\,MGA}$	$S_{10^{-8}\,MGA}$	
			100 DAS		
S_{6h}	287.70	329.32	357.11	321.70	327.98
S_{8h}	302.15	345.31	408.11	308.50	338.82
S_{10h}	300.20	318.41	376.18	303.15	318.97
Mean	296.67	331.73	372.91	308.33	
C.D. at 5%		C = 11.475	D = 13.421	C x D = 23.502	
			110 DAS		
S_{6h}	303.22	392.70	402.23	323.10	362.21
S_{8h}	322.51	404.65	411.21	382.21	381.32
S_{10h}	315.13	376.52	402.48	321.13	352.31
Mean	313.36	387.33	404.54	351.52	
C.D. at 5%		C = 11.134	D =13.381	C x D = NS	

N.B.: A similar common basal dose of 45 kg N + 35 kg P_2O_5/ha was applied to all pots.

Nitrate Reductase Activity

Soaking treatment $S_{10^{-6}\,M\,GA}$ provides higher value at all phases. Its effect followed by $S_{10^{-7}\,M\,GA}$ at each stage. Soaking with $S_{10^{-6}\,M\,GA}$ provide 73.14 and 31.34% more at 100 and 110 DAS respectively than S_W. Soaking duration S_{8h} provide more at all phases. Its effect equalled at 100 DAS and was followed on 110 DAS by S_{4h} and followed by S_{12h} at 110 DAS. S_{8h} provide 6.13 and 7.93% more at 100 and 110 DAS respectively than S_{12h}. $S_{10^{-6}\,M\,GA}$× S_{8h} provide more value at 100 DAS. Its effect followed by $S_{10^{-6}\,M\,GA}$ × S_{4h}, $S_{10^{-6}\,M\,GA}$ × S_{12h} and $S_{10^{-7}\,M\,GA}$ × S_{8h} at this phase. $S_{10^{-6}\,M\,GA}$ × S_{8h} provide 41.10% more than S_W× S_{4h} at the same phase. Interaction not affects that parameter at 110 DAS (*Table* 6).

Table 6: Impact of concentrations (C) and durations of pre-sowing seed treatment (D) of GA on seed yield per plant (g) of cultivar PUSA 334 of chickpea at harvesting time (120 DAS) (mean of four replicates)

Soaking durations (S_h)	Soaking concentrations ($S_{M\,GA}$)				Mean
	S_W	$S_{10^{-4}\,M\,GA}$	$S_{10^{-6}\,M\,GA}$	$S_{10^{-8}\,M\,GA}$	
S_{6h}	2.44	4.25	4.30	4.15	3.91
S_{8h}	2.55	4.34	4.58	4.29	3.99
S_{10h}	2.59	4.11	4.11	4.10	3.60
Mean	2.31	4.30	4.18	4.00	
C.D. at 5%		C = 0.140	D = 0.162	C x D = NS	

N.B: A similar common basal dose of 45 kg N + 35 kg P_2O_5 /ha applied in all plants.

Seed yield per plant

Soaking treatment $S_{10^{-6}\,M\,GA}$ provides the more value for seed yield. Its effect at par with $S_{10^{-7}\,M\,GA}$. Soaking with $S_{10^{-6}\,MGA}$ provide 85.29% more value than S_W Soaking duration S_{8h} provide the more value. Its effect at par with S_{4h}. S_{8h} provides 5.12% more value than S_{12h} which provide the lowest value. The interaction effect on the parameter not found significant (*Table* 7).

Table 7: Impact of concentrations (C) and durations of pre-sowing seed treatment (D) of GA on seed carbohydrate content (%) of cultivar PUSA 334 of chickpea at harvesting time (120 DAS) (mean of four replicates)

Soaking durations (S_h)	Soaking concentrations (S_{MGA})				Mean
	S_W	$S_{10^{-4}\,MGA}$	$S_{10^{-6}\,MGA}$	$S_{10^{-8}\,MGA}$	
S_{6h}	45.54	44.16	51.18	42.15	44.27
S_{8h}	44.23	51.14	52.35	41.19	46.18

Soaking durations (S_h)	Soaking concentrations (S_{MGA})				Mean
	S_W	$S_{10^{-4}\,MGA}$	$S_{10^{-6}\,MGA}$	$S_{10^{-8}\,MGA}$	
S_{10h}	39.52	42.55	48.15	44.50	42.65
Mean	43.10	45.12	53.12	42.22	
C.D. at 5%		C = 1.07	D = 1.30	C x D = 1.22	

N.B.: A similar common basal dose of 45 kg N + 35 kg P_2O_5 /ha was applied to all plants.

Seed protein and carbohydrate content

Soaking treatment $S_{10^{-6}\,MGA}$ provides more value for seed protein and carbohydrate content. Its effect followed by $S_{10^{-7}\,MGA}$. Soaking with $S_{10\text{-}6\,MGA}$ provide 26.13% and 32.14% more value than S_W respectively for protein and carbohydrate content. S_{12h} provide more value. Its effect at par with S_{8h}. S_{12h} provide 76.19% and 84% more value than lowest value providing soaking duration S_{4h} for protein and carbohydrate value respectively. $S_{10^{-6}\,MGA} \times S_{8h}$ provide more value for both. Its effect followed by $S_{10^{-6}\,MGA} \times S_{12h}$ and $S_{10\text{-}6M\,GA} \times S_{4h}$ for seed protein content and $S_{10^{-6}\,MGA} \times S_{12h}$ for carbohydrate content. $S_{10^{-6}\,MGA} \times S_{8h}$ enhanced the seed protein and carbohydrate content by 25.12% and 10.34% over $S_W \times S_{12h}$ and by 53.14% and 32.14% over low value providing $S_{10^{-5}\,MGA} \times S_{8h}$ respectively (*Table* 8 & 9).

Table 8: Impact of concentrations (C) and durations of pre-sowing seed treatment (D) of GA on seed protein content (%) of cultivar PUSA 334 of chickpea at harvesting time (120 DAS) (mean of four replicates)

Soaking durations (S_h)	Soaking concentrations (S_{MGA})				Mean
	S_W	$S_{10^{-4}\,MGA}$	$S_{10^{-6}\,MGA}$	$S_{10^{-8}\,MGA}$	
S_{6h}	16.10	17.37	23.70	16.40	17.69
S_{8h}	17.50	22.10	26.20	15.10	22.13
S_{10h}	18.74	21.10	22.25	18.13	21.58
Mean	17.55	18.36	21.35	18.14	
C.D. at 5%		C = 0.724	D = 0.842	C x D = 1.423	

N.B.: A similar common basal dose of 45 kg N + 35 kg P_2O_5 /ha applied in plants.

DISCUSSION

The performance of experiment assessed as SDW, NRA, and CAA and also SY as well as quality parameters viz., seed protein and carbohydrate content. The results discussed parameter-wise in the light of knowledge beam and researches carried-out by different other legume crop researchers below. The enhancing effect of application of GA at 100 and 110 DAS over the control on SDW / plant of cultivar, PUSA-334 receiving the systematically recommended amount of 45 kg N + 35 kg P_2O_5/ha traced to its different comparatively roles in pulses. For example, application of GA improves absorption and water use efficiency of elements (Tiwari *et al*., 2011), activity of biocatalysts (Mazid *et al*., 2016), cytokinesis, and cell enlargement (Taiz and Zeiger, 2010), chlorophyll content (Afroz *et al*., 2005), elongation of internode (Tiwari *et al*., 2011), membrane permeability (Taiz and Zeiger, 2010), P_N (Afroz *et al*., 2005), DNA and RNA and translation (Tiwari *et al*., 2011), and transport of sucrose (Ouzounidou and Ilias, 2005).

In view of its significant roles in several facets of crop physiology and very small quantity involved, it is reasonable to rope this wonderful PGR in innovative farm practices. The crop development depends chiefly on their capacity to reduce carbon in organs with chloroplasts by the functionality of the photosynthates for sink organs like roots etc. As the C fixing ability of plants is affected by nutrients among other factors, the availability of P and S to leguminous plants affects production and partitioning of photosynthates (Mazid and Khan, 2017; Mazid and Naz, 2017; Mazid and Khan, 2018). Further, it is gratifying to note that our experimental data confirmed beyond doubt the superiority of GA over water-sprayed control. Our findings reported similar with findings of previous researchers like Mobin *et al*. (2007). The growth enhancing impact of pre-seed priming treatment for 8 h with 10^{-6}M GA on the respective control on NRA and CAA studied at 100 and 110 DAS of PUSA-334 cultivar grown with basal dose of N and P could be described on the basis of its significance mentioned previously and the fact that the supply of PGR by seed priming would higher as compare to compensate the 'hidden hunger' of growing plants for gibberellic acid (Jafri, 2009; Thakare *et al*., 2011; Kumar and Mehera, 2022)).

Moreover, enhancement in SDW/plant of cultivar PUSA-334 contributed in enhancing the capacity of treated crops for biomass and nodule production. This is also manifested in SDW, confirmed by correlation workers

(Mazid, 2014). The enhancing impact of seed priming PGR over control on CAA and NRA of cultivar specifically PUSA-334, receiving the recommended dose of 45kg N + 35kg P_2O_5/ha, studied at 100 and 110 DAS is worth reported. The increase in CAA and NRA attributes to the PGR-induced enhancement in transcription/ translation of the active DNA which codes for CAA (Kumar *et al*., 2024) and NRA (Gawhale *et al*., 2024) to its significance in enhancing the permeability of membranes and absorption of elements (Roth-Benjerano and Lips, 1970; Sugiharto *et al*., 1992; Hopkins and Huner, 2009; Taiz and Zeiger, 2010; Kobagapu *et al*., 2019). These results are also in accordance with the data of previous authors (Premabatidevi, 1998) on NRA. The increasing impact of seed priming treatment for 8 h with 10^{-6}M GA over respective control on CAA and NRA of PUSA-334 cultivar of grown with the recommended amount of N and is a significant finding. The increase in the number of pods per plant and 110-seed weight resulting from the foliar application of GA in than control of chickpea cultivar PUSA-334 receiving basal dose of N and P is better mentioning.

Furthermore, the increase in the yield attributes may be traced to its various roles leading to observed higher values for SDW/ plant and, NRA and CAA of treated plants. In addition, it mediates differentiation (Afroz *et al*., 2005; Dharwe *et al*., 2019) leading to increased number of flowers that develop to pods. It plays role in cytokinensis and cell enlargement (Taiz and Zeiger, 2010; El-Eyuoon and Amin, 2018) resulting in proper development of under-developed pods especially at terminal end of branches; PN (Meena *et al*. 2024) supplying sufficient carbon skeleton; and membrane permeability (Crozier and Turnbull, 1984) and transport of sucrose (Khan, 2008) favouring partitioning hence more values for the yield attributes of treated plants (Shah and Samiullah., 2007). The increased yield attributing attributes of treated plants, especially pods/ plant and 100-seed weight contributed to the enhanced 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 (Mazid, 2014; Singh *et al*., 2022). The observed increase in seed protein and carbohydrate content due to pre-sowing seed treatment of GA is not wonderful. An enhancement in translation result from the application of PGR especially gibberellic acid, hence more values for seed protein value. GA mediating leaf expansion, Cori cycle, photosynthesis, respiration, N-fixation, T.C.A. cycle, glycolysis, B-oxidation, lipid metabolism and development of the cell organelles and synthesis of hydrolytic bio-catalysts, flower and fruit set, photosynthesis, translocation of food material (Tiwari *et al*. 2011; Fayaz *et al*., 2020). In addition, GA improves; absorption and NUE (use efficiency of nutrients, activity of enzymes, cellular enzymes, cell cycle and cell enlargement, DNA replication, cell reproduction, cell growth, chlorophyll content, elongation of Inter-node, P_N, nucleic acid DNA/RNA and translation, and transport of sucrose as well. Foliar application of GA_3 could have led to the observed improvement in plant height and LAI per plant of the treated plants (Singh *et al*. 2004).

Furthermore, GA_3 enhancing the rate of enzymes activities and absorption of nutrients responsible for improving the P_N, gs and Chl of treated plants and subsequently enhanced the content of Chl and leghaemoglobin. Moreover, higher levels of leghaemoglobin content also be responsible for enhanced content of Chl leading to higher P_N. The increase in the number of pods/ plant and 100-seed weight resulting from the foliar application of GA_3 than control (Mazid, 2014; Shah, 2008; Shah and Samiullah, 2007). The increase in the above yield attributes traced to its various roles leading to observed higher values for growth characters and, physiological and biochemical parameters of treated plants. Also, it mediates differentiation leading to enhanced number of flowers which develop into pods. It plays role in cell division and cell enlargement resulting in proper development of under-developed pods especially at the terminal end of branches; P_N supplying sufficient carbon skeleton; and membrane permeability and transport of photosynthates favoring partitioning hence more values for the SY of treated plants (Singh *et al*., 2004; Taiz and Zeiger, 2010).

In addition, chickpea considered valuable for enhancing soil fertility in agricultural systems because of their capacity to add N to the soil. A net N benefit will occur only when the total amount of nitrogen fixed in plant material exceeds the quantity of nitrogen removed. The augmenting effect of pre-sowing seed treatment with 10^{-6}M GA_3 for 8h over control treatment on pods per plant and seeds per pod and of spray treatment at 100 and 100 DAS with the same concentration of GA_3 in comparison with the control on these characters as also on 100-seed weight is understandable (Jafri, 2009; Dharwe *et al*., 2019). The improvement in growth, physiological, biochemical and metabolic parameters which resulted from the spray of these nutrients together with enhancement in differentiation may lead to the improvement in yield characters, hence maximum values for pods/ plant, seeds/ pod and 100-seed weight, BY and HI (Dharwe *et al*. (2019). Thus, plant height, pods per plant, seeds per pod, 100 seed weight and HI appeared as main yield factors in chickpea. GA_3 has definite role to play in the number of flowers produced and maintenance. The increased seed yield may be also due to the application of P, S together with GA_3 have helped the flowering process (Dharwe *et al*., 2019; Singh *et al*., 2022; Fayaz *et al*., 2020; Kumar *et al*., 2024; Gawhale *et al*. 2024)). The observed increase in seed protein and carbohydrate content due to seed priming treatment of GA_3 is not wondering hence higher values for seed protein and carbohydrate content (Taiz and Zeiger 2010).

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CONCLUSION

All parameters reported significant and promoted by the soaking with different levels of GA, however, with highest stimulation reported for a 10-h soaking treatment (duration) with 10^{-6}MGA. The total seed protein and total carbohydrate content stimulated much greatly by this approach and results are better than still any other approach applied for chickpea growth and quality values enhancement.

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