

Effect of irrigation, phosphorous and potassium application on root traits, soil microbial growth and physiology of green gram

ABSTRACT

Aims: To manage the implication of irrigation water at different growth stages coupled with basal phosphorus and foliar potassium application targeting to reduce water stress.

Study design: Split split-plot design.

Place and Duration of Study: Agricultural Research Station, Chatabar, Odisha. India, during 2022-2023 cropping season.

Methodology: Three irrigation treatments in main plots (I1, I2 and I3) - I1 included three irrigations at vegetative, flowering and pod formation periods, I2 skipped one irrigation at pod formation and I3 skipped at flowering. Sub-plot consisted of phosphorus fertilizer treatments (P1, P2 and P3) providing 100, 85 and 115% recommended dose of P. In sub sub-plots- 2% foliar spray of KCl at vegetative and flower initiation periods. The parameters recorded soil moisture depletion, root traits, microbiological parameters, plant physiological parameters and grain yield.

Results: The depletion of soil moisture increased with crop growth; I3 depleted at a maximum rate (4.5 mm d^{-1}) followed by I2 (4.1 mm d^{-1}). The enhanced availability of root zone soil moisture improved microbial biomass carbon (MBC) and dehydrogenase activity (DHA). The I1 improved the maximum root tips, forks and crossings; while I3 reduced an overall of 131% of the root traits than I1. The crop growth rate (CGR) and leaf area index (LAI) were also improved according to soil moisture availability; however, I2 merely decreased 8 and 13% in CGR and LAI as compared to I1. P3 improved the maximum of the soil microbiology (MBC and DHA), root improvement and plant physiology. I1P3 produced the highest grain yield of 9.06 q ha^{-1} , although, I2P3 produced was just 7% lower than I1P1.

Conclusion: The application of three irrigation produced maximum grain yield; while the skipping of one irrigation at pod formation stage with 15% higher P application could provide satisfactory yield where availability of irrigation water is limited.

Keywords: Soil moisture, crop growth, microbial biomass carbon, root traits, deficit irrigation

1. INTRODUCTION

Green gram (*Vigna radiata* L.), well known as 'moongbean' or 'moong' of the family 'Leguminosae', is under cultivation since prehistoric time in India. It serves as a major source of dietary protein and an excellent source of carbohydrates, essential fatty acids, vitamins, minerals and fiber for the vast majority of people [1]. Green gram is mainly cultivated in East Asia, South East Asia and Indian subcontinent. India is the largest producer (25% of global production), consumer (27% of world consumption) as well as importer (14%) of pulses in the world [2]. The important green gram producing states in the country are Rajasthan followed by Karnataka, Maharashtra, Madhya Pradesh, Andhra Pradesh, Gujarat, Rajasthan Bihar and Odisha. It is grown on about 40.38 lakh hectares with a total production of 31.5 lakh tonnes with a productivity of 783 kg/ha and contributes 11 % to the total pulse

28 production in the year 2021-22 [3]. Although, pulses production in India has not kept up with
29 growth in demand calling for import. Scarcity of irrigation water or poor management of its
30 cause the reduce production of pulses. India is not a water-poor country but due to the
31 growing human population, severe neglect and over-exploitation of this resource, water is
32 becoming a scarce commodity [4]. India's groundwater resources are rapidly depleting,
33 especially in the northwest as the bulk of it being used for irrigation. In the dry season water
34 stress on agricultural dryland as in West and East India has forced farmers to be idle to
35 cultivate their land due to the increasing risk of crop failure [5]. Therefore, dryland
36 optimization for agricultural uses needs to be supported by an appropriate irrigation
37 technology alternative. Water stress affects plant physiology especially the roots.

38 Soil moisture is the key factor restricting plant growth and development, and the
39 most important factor affecting vegetation development. Depletion of soil moisture increase
40 the penetration resistance of soil which affects the development of roots and its functions [6].
41 In this harsh condition, an alternative plant strategy may be to stimulate water uptake not by
42 increasing the total mass of root material, but by producing finer roots with relatively greater
43 length and surface area per unit mass. Green gram is known for its tolerance to adverse
44 environmental condition, root development during its growth period that is also notably
45 affected by the moisture content of the soil. Because of that green gram has been chosen for
46 the test crop. In addition, phosphorus (P) is one of the essential macronutrients for plant
47 growth and development, and it is an integral part of the major organic components,
48 including nucleic acids, proteins and phospholipids [7]. In current situation, although total P
49 is abundant in most soils, a large proportion of P is fixed by soil mineral components (e.g.,
50 aluminum or iron) into insoluble chemical complexes that are not readily accessible to plants
51 [8]. Therefore, low P availability is considered as a major limiting factor for plant growth,
52 development and yield in more than 60% of the world's arable land [9]. However, only 10–
53 30% of the P in P fertilizers are estimated to be used by plants [8]. Local P deficiency
54 appears to be the external driver of primary root growth inhibition, promoting lateral root
55 formation and increasing the production of root hairs [10]. Moreover, the effect of the water
56 driven stress in plant physiological aspect can be addressed by nutrient management like
57 potassium (K) that can improve the tolerance of crop plants to various types of abiotic
58 stresses, and it also improved subsequent growth and yield. Potassium plays an important
59 role in combating the adverse effect of water stress through its effect on different
60 physiological process. The availability of potassium to the plant decreases with decreasing
61 soil water content, due to the decreasing mobility of potassium under these conditions.
62 There is lack of data regarding the development of roots morphology in water scarce
63 condition under field situation.

64 Considering all the aspect of judicious water management, P utilization through plant
65 roots and stress alleviating role of K, an experiment was formulated considering skipping of
66 one irrigation in critical stage as main plot, improvement of basal P fertilizer application and
67 foliar application of K as sub-plots for the growing of green gram. The objectives of the
68 present study were- 1) assessment of the **pattern regarding the** depletion of root zone soil
69 moisture, 2) the impact on root growth and soil microbiological activities **under irrigation, P**
70 **and K** management and 3) the influence of the management **options** on the plant physiology
71 and grain yield.

72 **2. MATERIAL AND METHODS**

73 **2.1. Experimental site description**

74 The study was conducted at Agricultural Research Station, Chatabar, Odisha. The climate is
75 hot, humid subtropics with an average annual rainfall of approximately 1490 mm. The mean
76

77 annual minimum and maximum temperatures were of 25.5 and 32.3 °C, respectively and for
78 the green gram season, they were 13.4 and 27.3 °C, respectively. The rainfall received
79 during green gram growing period was 22.9 mm.

80 **2.2. Soil properties of the experimental site**

81 A number of soil cores were extracted from the experimental plot to a depth of 450 mm to
82 characterize the soil physical as well as chemical properties. The detail analytical
83 procedures were elaborated later on in this material and methods.

84 The basic physical and chemical soil properties at the time of green gram sowing at
85 150 mm depth increment indicating that the soil was sandy clay loam to sandy loam in
86 texture with water holding capacity (WHC) of 43 to 51% along with a slightly acidic in soil pH
87 (6.2 to 6.3). The bulk density increased from 1.3 to 1.4 Mg m⁻³ with increasing soil depth.
88 The field capacity and permanent wilting point ranged from 23 to 27% and 12 to 14%,
89 respectively. The soil had low to medium range of soil organic matter (2.4 – 3.6 g kg⁻¹), low
90 in available nitrogen (107 - 152 kg ha⁻¹), available phosphorus (7 - 10 kg ha⁻¹) and available
91 potassium (82 - 112 kg ha⁻¹).

92 **2.3. Experimental details**

93 Before starting of the experimental, the field was limed and thoroughly tilled and kept as
94 such for two weeks for completion of liming reactions. Thereafter plots (3 × 4 m) were
95 demarcated and buffer channels (1m) were made for irrigation treatment purpose. The
96 experiment was delineated in double split plot design. Three irrigation treatments in main
97 plots were proposed- I1, I2 and I3. I1 provided three irrigations at vegetative, flowering and
98 pod formation periods, I2 skipped one irrigation at pod formation and I3 skipped at flowering.
99 Sub-plot consisted of phosphorus fertilizer treatments- P1, P2 and P3; where P1 provided
100 100% recommended dose of P, P2 supplied 85% and P3 supplied 115% of the
101 recommended dose of P. In sub sub-plots, there were 2% foliar spray of KCl at vegetative
102 and flower initiation periods. The recommended dose of N, P₂O₅ and K₂O for green gram
103 crop were 25, 40 and 25 Kg ha⁻¹.

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105 **2.4. Observations**

106 **2.4.1. Soil moisture depletion rate**

107 Screw auger was used for collection of fresh soil sample from the depth of 0 to 40 cm for
108 gravimetrically measurement of soil moisture determination. The amount of depth soil
109 moisture was derived by multiplying gravimetric moisture with bulk density and soil depth.
110 Thereafter, the depletion rate (mm d⁻¹) of soil moisture calculated as-

$$111 \text{ Depletion rate} = \frac{(V_1 - V_2)}{(T_2 - T_1)} \quad (1)$$

112 Where, V1 and V2 were depth moisture content (in mm) at T1 and T2 days after sowing.

113 **2.4.2. Soil chemical parameters**

114 Field moist soil samples were collected in triplicate from each of the treatment plot at a depth
115 of 0-450 mm at 150 mm depth interval with a auger. They are pooled together to make a
116 composite sample. Bulk samples were taken to the laboratory in bags. The samples were
117 then allowed to air dry for 72 hr before chemical and physical analysis. The air-dried
118 subsamples of each sample were then hand crushed, passed through 2 mm sieve and was

119 stored for determination of various physical and chemical analyses. The pH of the soil was
120 determined by using soil and water ratio 1:2.5 (w/v) [11] and glass electrode pH meter
121 (Systronix, Hyderabad, India). Oxidisable organic carbon (C_{ox}) of the soil was determined
122 following the method of Walkley and Black [12]. The available N was determined by Kjeldahl
123 flask using alkaline permanganate method [13] following titration with H_2SO_4 . Available
124 phosphorus content was determined following Bray-1 method [14]. Available K_2O was
125 estimated by neutral normal ammonium acetate using flame photometer [11].

126 **2.4.3. Root parameter**

127 Root samplings were made following auger methods. Root samples were collected to a
128 depth of 450 mm sub-dividing the soil cylinder in three sub-samples (0–150, 150–300, 300–
129 450 mm) and washed. Different root parameters viz, root tips, root forks and root crossings
130 were determined by the image analysis software “WinRHIZO” [15].

131 **2.4.4. Crop growth rate (CGR)**

132 Crop growth rate (CGR) is the rate of dry biomass production per unit ground area per unit
133 time [16]. It was calculated by using the following formula and expressed as $g\ m^{-2}\ day^{-1}$.

$$134\ CGR = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{1}{A} \quad (2)$$

135 where, W_1 , the dry weight of the plant ($g\ m^{-2}$) at time t_1 ; W_2 , the dry weight of the plant ($g\ m^{-2}$)
136 at time t_2 ; $(t_2 - t_1)$, the time interval in days; A, the unit land area (m^2). The dry biomass was
137 measured at the vegetative, flowering and at pod-formation stages of the crop.

138 **2.4.5. Leaf area index (LAI)**

139 The green leaf portions were separated and the area of the leaves was measured. Mean
140 value per plant was used in calculating the leaf area index (LAI) which was derived using the
141 formula:

$$142\ LAI = \frac{\text{Measured leaf area per plant (m}^2\text{)} \times \text{no. of plants}}{\text{Ground area (m}^2\text{)}} \quad (3)$$

143 **2.4.5. Yield**

144 The crops were harvested manually simply by uprooting at grain maturity stage and allowed
145 to dry in the threshing yard. After complete sun drying, when the soil moisture content of the
146 nearly 15%, the crop was threshed by beating with wooden sticks. The seeds were
147 winnowed, cleaned and seed weight was recorded and final yield was converted into $kg\ ha^{-1}$.

148 **2.5. Statistical analysis**

149 The analysis has been done with STAR (Statistical Software for Agricultural Research)-IRRI
150 (International Rice Research Institute). The first factor is tillage which was the main plot and
151 the second factor is the mulching. Duncan’s Multiple Range Test (DMRT) was performed to
152 compare the treatments at 5% level of significance.

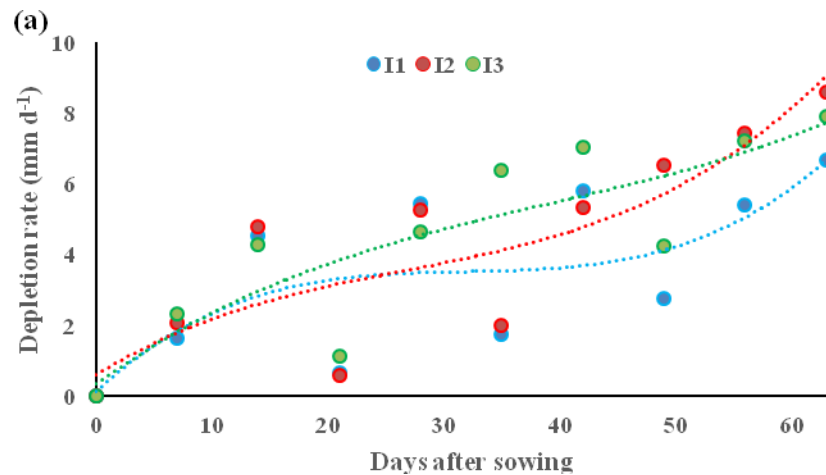
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154 **3. RESULTS AND DISCUSSION**

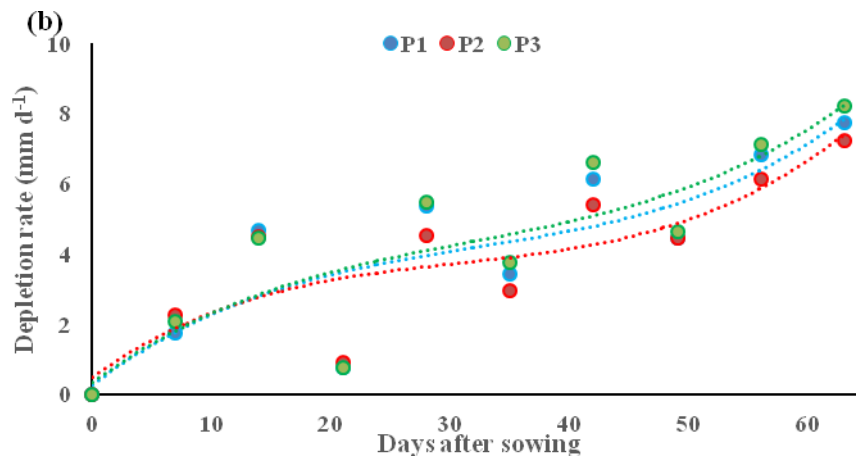
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156 **3.1. Depletion of soil moisture**

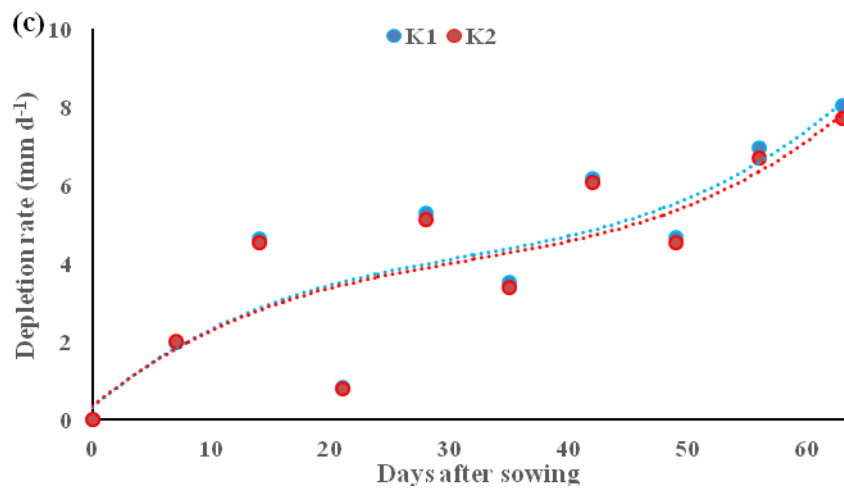
157 Initially the amount of soil moisture was similar for all the treatments; thereafter treatments
158 changed the depletion pattern of root zone soil moisture either through transpiration of plant
159 canopy or the evaporation from soil surface as modified by foliage cover over soil surface.
160 The depletion rate of soil moisture was around 1.7 to 2.2 mm d⁻¹ during initial period of crop
161 growth and hence showed no significant differences among irrigation treatments. While with
162 the advancement of crop growth, depletion sequences changed significantly (**Figure 1**). I3
163 resulted in the maximum depletion rate from vegetative (4.8 mm d⁻¹) to reproductive period
164 (5.7 mm d⁻¹); where I2 increased depletion rate at the later period of crop growth with
165 averaged of 6.5 mm d⁻¹ (**Figure 1.a**). Although I1 showed higher depletion rate over I2 at the
166 initial and vegetative period, that decreased below 18 to 37% than I2 during flowering to
167 maturity period of green gram. The higher amount of soil water in the root zone due to the
168 supply of water through irrigation under I1 accentuated the depletion rate through the root
169 growth or direct evaporation. Mukherjee et al. [17] similarly explained higher depletion of soil
170 moisture in working with chickpea. While the elevated depletion rate under I3 can be
171 explained by the observation of Bhattarai et al. [18] who noticed higher depletion of moisture
172 where less irrigation had been received and plant extracted the soil water to its fullest extent.
173 Regarding the application of P, the maximum depletion rate was observed under P3 followed
174 by P1 and the least was under P2 with average depletion rate of 4.5, 4.2 and 3.7 under P3,
175 P1 and P2, respectively (**Figure 1.b**). The application of P fertilizers significantly increased
176 the stomata density and stomatal conductance which pronounced the transpiration activity
177 resulted more depletion of soil moisture [19]. However, application of foliar spray of K
178 secured no significant marks on soil moisture depletion.



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Fig.1. The depletion rate of soil moisture under different (a) irrigation, (b) phosphorus and (c) potassium treatments.

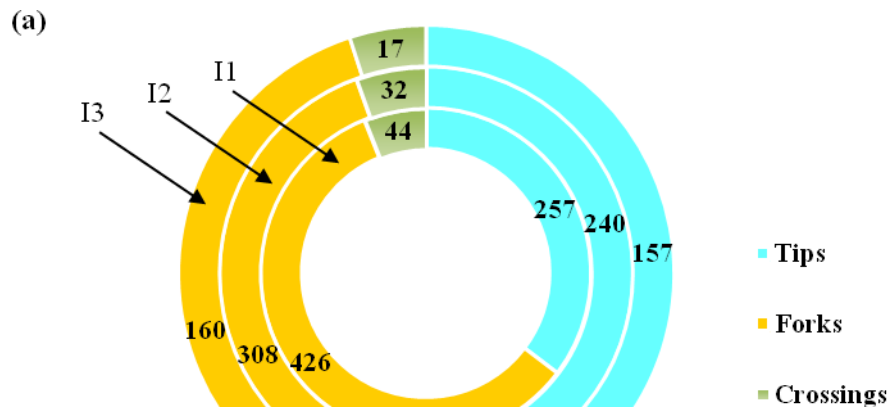
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186 **3.2. Root system modification**

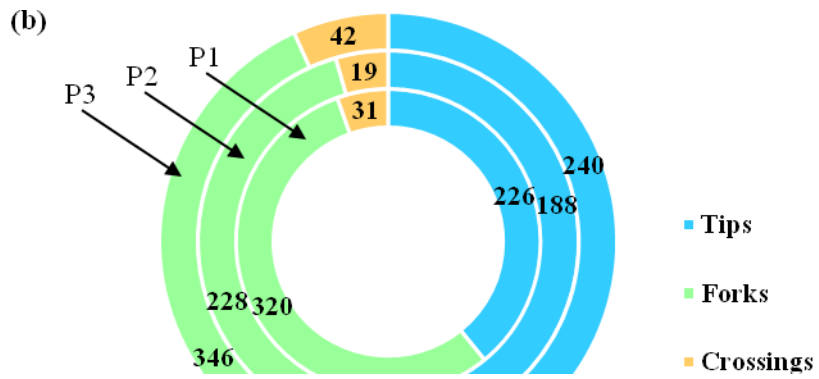
187 The changes of soil moisture storage happened due to varied depletion pattern modified the
188 amount of root tips, forks and crossings. Root tips, fork and crossings play a significant role
189 in resource acquisition mainly for capturing water and nutrients more efficiently from deep
190 inside the soil. The root tips, forks and crossings as shown in **Figure 2(a-c)** were distributed
191 differently with the irrigation treatments. Figure shows the relative proportion of root tips,
192 forks and crossings in three irrigation treatments. The proportion of root tips was maximum
193 and crossings was the least. I1 resulted in the maximum amount of root tips, forks and
194 crossings followed by I2 (**Figure 2.a**). The least developed roots were observed under I3.
195 Naruse et al. [20] argued that due to the field irrigation condition root traits responded early
196 and exhibited increased root tips, forks and crossings. That helped plant in water acquisition
197 as it is strongly lined with the spatial distribution of water in soil [21].

198 P also improved the root tips, forks and crossings significantly. P3 produced
199 significantly 6, 8 and 35% higher root tips, forks and crossings as compared to P1. P2
200 resulted in the lowest amount of the root traits (**Figure 2.b**). Similarly, Hodge et al. [22]
201 stated that plants modify their root architectural traits like reduction of primary roots, lateral
202 roots and root tips under low P conditions. Where, foliar K application showed no significant
203 changes in these parameters (**Figure 2.c**).
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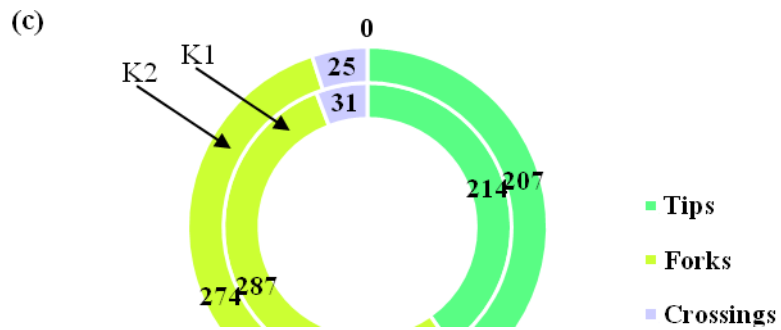
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Fig. 2. The distribution of the amount of root tips, forks and crossings under different (a) irrigation, (b) phosphorus and (c) potassium treatments.

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212 **3.3. Microbial biomass carbon and dehydrogenase activity**

213 The microbial biomass carbon (MBC) was observed at the critical growth periods of green
214 gram where MBC values were increased from vegetative to reproductive stages and
215 thereafter it reduced to the lowest value at maturity period. MBC denotes the portion of soil
216 that is responsible for energy transformation, nutrient cycling and organic matter
217 transformation. Irrigation treatments effectively influenced the MBC. Although, initially, so
218 significant differences were noticed among the treatments; I1 resulted in the maximum MBC
219 of $264 \mu\text{g g}^{-1}$ followed by I2 which was 6% lesser than I1 during reproductive period (**Figure**
220 **3.a**). I3 produced the least MBC at reproductive period but I3 improved 66% higher MBC
221 than I2 at maturity of green gram. Velmourougane et al. [23] also observed strong positive
222 correlation of MBC with soil moisture content in their study. In case of P treatments, P3
223 resulted in the maximum MBC throughout the growing period followed by P1 and the least
224 amount of MBC was resulted at P2. Similar result was found by Bolat et al. [24].

225 The trend of dehydrogenase activity (DHA) of soil was observed comparable as
226 observed in MBC under irrigation, P and K treatments (**Figure 4**). I1 secured the maximum
227 DHA followed by I2 and I3 resulted the lowest DHA of all. While in case of P treatments the
228 sequence followed as $P3 > P1 > P2$. The K application showed no significant changes.

229 **3.4. Plant physiology and grain yield**

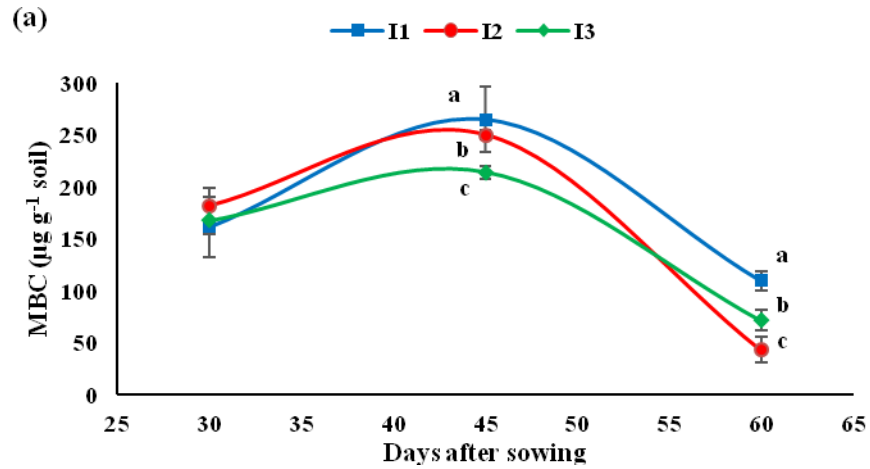
230 The influence of irrigation and nutrient treatments impacted physiology of green gram. The
231 leaf area index (LAI) under different irrigation treatments has been depicted in **Table 1**. It
232 was observed that LAI improved continuously with the advancement of crop growth and
233 maximum was achieved at maturity period. I1 produced the maximum LAI which was 13%
234 higher than I3 at reproductive period and also 14 and 31% higher than I2 and I3, respectively
235 at maturity period of green gram. Improvement of LAI under sufficient supply of soil moisture
236 was previously reported by Nandi et al. ([25] in working with lentil. LAI showed the maximum
237 improvement under P3 and lowest improvement under P2. K showed no significant impact
238 on LAI development. Koneni [26] observed the application of phosphorus in the increment of
239 LAI in in green gram over its non/less application.

240 Crop growth rate (CGR) showed clear decrement with the crop development. The
241 average CGR at vegetative, reproductive and maturity periods were $7.0, 2.9$ and $1.5 \text{ g m}^{-2} \text{ d}^{-1}$,
242 respectively. I1 and I2 produced maximum CGR up to reproductive period; thereafter CGR
243 of I2 decreased 39% from I1; while I3 resulted in the lowest CGR during later growth period.
244 Improvement of CGR through the application of P was quite significant in due course of crop
245 growth. The reduced values of CGR were subjected to water as previously reported by
246 Bandyopadhyay et al. [27] in their research regarding moisture driven stress physiology
247 changes. P3 resulted in significantly 10 and 24% higher CGR at vegetative; 19 and 38%
248 higher CGR at reproductive and 14 and 54% higher CGR at maturity periods over P1 and
249 P2, respectively. However, K showed no significant changes in CGR of green gram. P's
250 growth improvement was related with its role in synthesis of energy-rich compounds (ATP,
251 CTP, GTC), protein synthesis, biochemical adaptations, roots-shoot development etc [28].

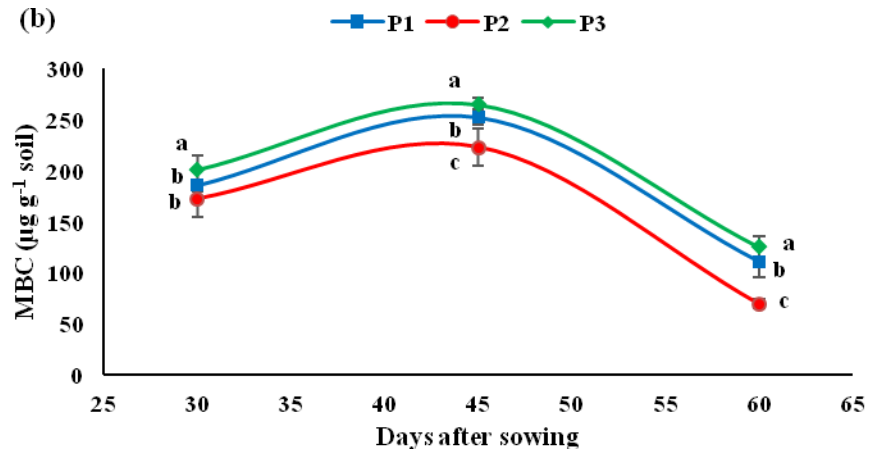
252 Grain yield of green gram under different irrigation, P and K treatments has been
253 presented in **Table 1**. I1 produced the maximum grain yield which was 18 and 49% higher
254 than I2 and I3. While P3 produced the highest yield of 7.21 q ha^{-1} which was 12% superior of
255 P1. K's role in grain yield improvement was non-significant. I1P3 produced the highest grain
256 yield of 9.06 q ha^{-1} followed by I1P1 (8.03 q ha^{-1}). Although, I2P3 produced 7.47 q ha^{-1}
257 grain yield which was merely 7% lower than I1P1. I3P2 secured the least grain yield of 3.11
258 q ha^{-1} .

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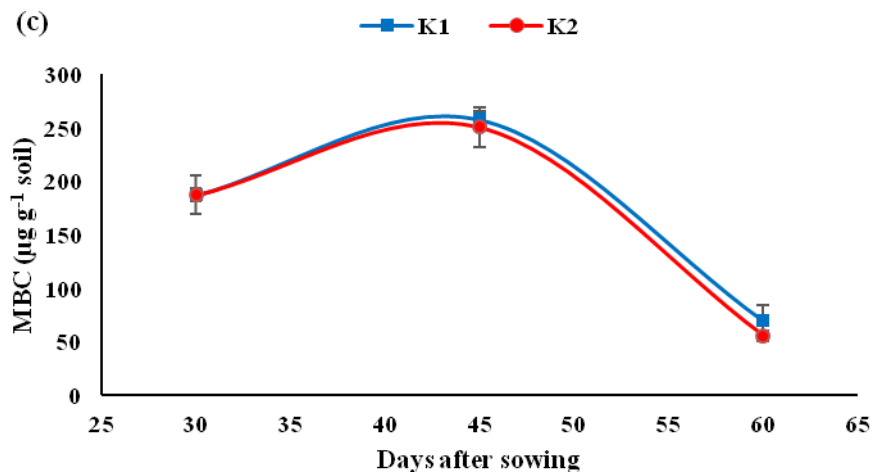
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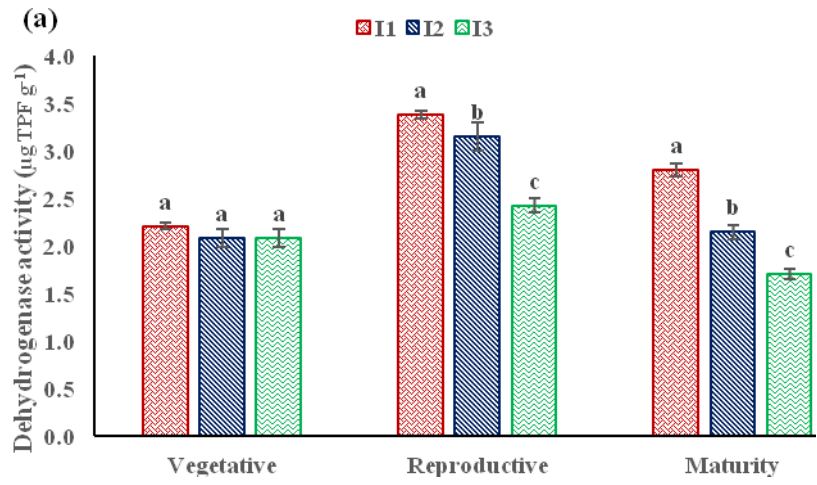
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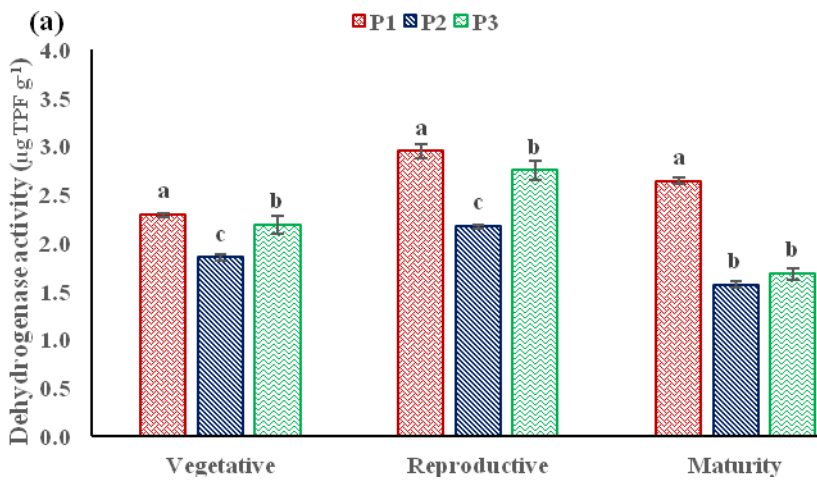
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Fig.3. Soil microbial biomass carbon (MBC) under different (a) irrigation, (b) phosphorus and (c) potassium treatments (Different lowercase are significantly different at $p < 0.05$ according to DMRT test).

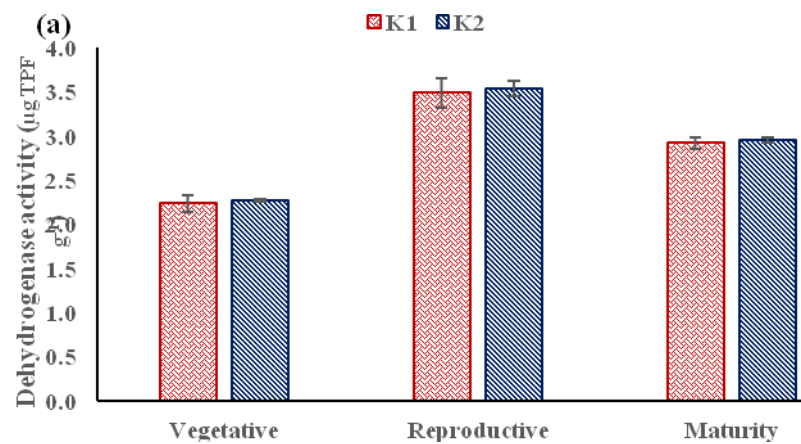
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Fig.4. Dehydrogenase activity in soil under different (a) irrigation, (b) phosphorus and (c) potassium treatments (Different lowercase letters within columns are significantly different at $p < 0.05$ according to DMRT test).

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Table 1. Leaf area index, Crop growth rate ($\text{g m}^{-2} \text{d}^{-1}$) and Yield (kg ha^{-1}) of green gram under different irrigation, phosphorus and potassium treatments

	Leaf area index			Crop growth rate ($\text{g m}^{-2} \text{d}^{-1}$)			Yield (kg ha^{-1})
	Vegetative	Flowering	Pod formation	Vegetative	Flowering	Pod formation	
Irrigation (I)							
I1	1.54 ^a	2.15 ^a	2.74 ^a	7.17	3.29 ^a	1.91 ^a	716.0 ^a
I2	1.40 ^b	2.12 ^a	2.41 ^b	6.84	3.09 ^b	1.37 ^b	606.5 ^b
I3	1.40 ^b	1.90 ^b	2.12 ^c	6.85	2.21 ^c	0.88 ^c	478.6 ^c
LSD ($p < 0.05$)	0.11*	0.15*	0.19*	ns	0.16*	0.23*	87.0**
Phosphorus (P)							
P1	1.37 ^b	1.97 ^a	2.34 ^b	6.95 ^b	2.85 ^b	1.67 ^b	642.4 ^b
P2	1.16 ^c	1.76 ^b	1.92 ^c	6.21 ^c	2.41 ^c	1.24 ^c	437.6 ^c
P3	1.46 ^a	2.09 ^a	2.51 ^a	7.69 ^a	3.35 ^a	1.91 ^a	721.1 ^a
LSD ($p < 0.05$)	0.15*	0.10*	0.23*	0.15*	0.27*	0.21*	13.8*
Potassium (K)							
K1	1.42	2.04	2.42	7.39	2.76	1.49	606.9
K2	1.34	2.00	2.31	7.21	2.64	1.28	593.9
LSD ($p < 0.05$)	ns	ns	ns	ns	ns	ns	ns
Interactions							
I*P	*	**	*	ns	*	*	**
I*K	ns	ns	ns	ns	ns	ns	ns
P*K	ns	ns	ns	ns	ns	ns	ns
I*P*K	ns	ns	ns	ns	ns	ns	ns

277

278 **4. CONCLUSION**

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280 The application of three irrigation and 15% excess P improved the soil moisture depletion
281 rate, root morphological traits, soil microbiological behaviour and plant physiology of green
282 gram in the maximum extent. However, the skipping of one irrigation at pod formation stage
283 with 15% higher P application significantly impacted on soil moisture scenario, soil
284 microbiology and plant growth. So, it can be concluded that though the application of three
285 irrigation produced maximum grain yield, skipping of one irrigation at pod formation stage
286 with 15% higher P application could provide satisfactory yield where availability of irrigation
287 water is limited.

288

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292 the present research.

293

294 **COMPETING INTERESTS**

295 The authors declare that they have no known competing interests.

296 **AUTHORS' CONTRIBUTIONS**

297

298 All authors shared their views in the process of formulation of the experiment and making of
299 the draft. Field investigation, data collection and formal laboratory analysis were performed
300 by J. Jena and M. Layek; R. Nandi has written the first draft of paper and curation of the
301 data. G.H. Santra and G. Sahu put valuable inputs in the improvement and representation of
302 the manuscript.

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