

# EFFECT OF TRANSIENT WATERLOGGING STRESS ON GROWTH, PHYSIOLOGY AND YIELD OF COWPEA (*Vigna unguiculata* L.) IN SEMI-ARID REGION

## ABSTRACT

Cowpea cultivation during rainy season is highly affected by the waterlogging stress due to unpredicted high-intensity rains. The studies on assessment of waterlogging effect on different growth stages of cowpea are necessary for planning mitigation strategies. Hence study was conducted during *kharif* (June to September) 2022 under factorial randomized block design (FRCBD) set up. The first factor consists of seven waterlogging durations (3 to 15 days), and second factor was three growth stages of cowpea (15 DAE; Days after emergence, 25 DAE and at 50% flowering). The results revealed that regardless of growth stages, growth and yield attributes were drastically decreased with increased duration of waterlogging. The highest plant height (25.07 cm), number of branches plant<sup>-1</sup> (5.33) and leaf area (205.27 cm<sup>2</sup> plant<sup>-1</sup>), and number of pods plant<sup>-1</sup> (4.27), pod length (15.24 cm), number of seeds pod<sup>-1</sup> (14.27), grain (6.27 g plant<sup>-1</sup>) and haulm yield (15.62 g plant<sup>-1</sup>) were recorded with 3 days of waterlogging, whereas, lowest values were reported with 15 days of waterlogging. Regarding growth stages, highest growth, and yield attributes were recorded with waterlogging during 50% flowering, followed by 25 DAE and 15 DAE. Moreover, the correlation study indicated that physiological parameters such as leaf protein content ( $r = 0.95$ ) and Normalized Difference Vegetation Index NDVI ( $r = 0.97$ ) were positively related to grain yield. It was found that, cowpea is sensitive to high-intensity waterlogging (beyond 3–5 days) especially during the early growth stage (15 DAE).

**Keywords:** Cowpea; growth stages; protein; proline; waterlogging; yield.

## 1. INTRODUCTION

Cowpea (*Vigna unguiculata* L.) belonging to the family fabaceae acts as a source of livelihood for farmers in the semi-arid region [1]. Being vegetable meat, cowpea is a rich source of protein, fiber, amino acids, antioxidants, folic acid, phenols and other essential minerals [2]. It is cultivated mainly in countries of semi-arid regions such as Africa, India and Sri Lanka. Globally cowpea is cultivated in an

area of around 15 m ha, with production of 9 m t and productivity of 6 q ha<sup>-1</sup> [3]. It is mostly cultivated under rainfed conditions in dry regions as it facilitates shorter life cycle with less water requirement [4]. In India, cowpea is cultivated in an area of 1.6 lakh ha, with the production of 1.03 lakh tonnes and productivity of 8 q ha<sup>-1</sup>. It is a minor pulse crop cultivated mainly in the states of Andhra Pradesh, Karnataka, Tamil Nadu, Telangana, Mizoram, Nagaland and Tripura. The major area (1.01 lakh ha) of cowpea cultivation is under rainfed conditions during *kharif* [5]. Increased summer rainfall saturates the soil before sowing. Waterlogging due to frequent higher intensity rainfall during *kharif* affects the cultivation of cowpea, causing reduction in growth and yield. Waterlogging alters the soil physical and electrochemical properties. It reduces the oxygen diffusion in the soil by 10000 times causing hypoxia and anoxia stress to plants. It also reduces nutrient uptake due to reduced root activity [6].

The associated stress tolerance mechanisms protect the plants from waterlogging effects up to certain duration. The recent study by Basavaraj et al. [7] indicated that waterlogging for 10 days during the early seedling stage reduced the cowpea grain yield by 39.18%. Moreover, Olorunwa et al. [8] indicated that 10 days of waterlogging during vegetative stage reduced the cowpea grain yield by 76%. Whereas according to a previous study by Umaharan et al. [9], waterlogging throughout the crop cycle decreased cowpea grain yield by 53%, while waterlogging at the reproductive stage did not affect yield. Stress tolerance of crops is highly reliant on the genotype, ecotype and adaptive mechanisms. The impact of waterlogging on different growth stages of cowpea remains unclear. The study on the effect of varied durations of waterlogging stress during different growth stages aids in the development of mitigation strategies to obtain higher yield of cowpea. Hence this research was carried out with the objectives to i) assess the effect of varied durations of waterlogging during different growth stages on growth and yield of cowpea, and ii) examine the effect of waterlogging on selected physiological parameters of cowpea and its relation with yield.

## 2. MATERIALS AND METHODS

The experiment was conducted during *kharif* 2022 at the ICAR-National Institute of Abiotic Stress Management, Baramati, Maharashtra, India. The site is located under the scarcity zone of Maharashtra (ACZ-95) with hot and semi-arid climate (AER-6). The experiment was conducted in the pots (capacity: 14 kg soil volume) under factorial randomized block design (FRCBD) with three replications. The durations of waterlogging (D) (D<sub>1</sub> – 3, D<sub>2</sub> – 5, D<sub>3</sub> – 7, D<sub>4</sub> – 9, D<sub>5</sub> – 11, D<sub>6</sub> – 13 and D<sub>7</sub>

– 15 days) were considered as the first factor and time/stages of waterlogging (T) [T<sub>1</sub> – 15 DAE (Days after emergence), T<sub>2</sub> – 25 DAE and T<sub>3</sub> – at 50% flowering stage] were considered as the second factor. Clayey textured soil black was used for the study. The soil was alkaline in reaction (pH: 8.50) with normal electrical conductivity (0.29 dS m<sup>-1</sup>), low in available nitrogen (107.87 kg ha<sup>-1</sup>) and phosphorus (8.16 kg ha<sup>-1</sup>), and medium in available potassium (180.0 kg ha<sup>-1</sup>) and organic carbon content (0.51%). The cowpea variety DC 15 obtained from the University of Agricultural Sciences, **Dharwad** was sown on 25<sup>th</sup> July 2022. The fertilizer requirement per pot was calculated (Urea: 73.87 mg, DAP: 0.68 g and MOP: 0.25 g) and applied 100% as basal dose on the soil volume basis. Cowpea plants were subjected to waterlogging stress for varied durations at different growth stages by keeping the pots in the constructed concrete tank and maintained the water level of 2.5 cm above soil, **whereas**, the control pots were maintained with similar management practices except waterlogging stress, where the irrigation was scheduled regularly at 60% of field capacity. The plant growth, physiological and yield parameters were recorded during the course of experiment. The leaf proline content at stress was determined as per the method given by Bates et al. [10] and the leaf protein content was determined as suggested by Singleton et al. [11]. The relationship between plant physiological parameters and yield was studied through Pearson's correlation coefficient analysis. The experimental data were subjected to statistical analysis as outlined by Gomez and Gomez [12]. The critical difference (**P = 0.05**) was worked out wherever 'F' test was found significant. Further the mean value of all factors and their interactions were separately subjected to Duncan Multiple Range Test (DMRT) using the corresponding error mean sum of squares and degrees of freedom. Control treatment was **analyzed** by following Randomized Complete Block Design (RCBD) and presented in the table.

### **3. RESULTS AND DISCUSSION**

#### **3.1 EFFECT OF WATERLOGGING ON GROWTH PARAMETERS OF COWPEA**

The key growth parameters at 60 days after sowing (DAS) and at harvest were significantly affected by varied durations of waterlogging during the different growth stages (**Table 1**).

##### **3.1.1 Plant height (cm)**

Among the durations of waterlogging (D), significantly the highest plant height at 60 DAS (20.86 cm) and harvest (25.07 cm) was recorded with 3 days ( $D_1$ ) of waterlogging, whereas the lowest plant height was recorded with 15 days of waterlogging ( $D_7$ ). Similarly, among the time/stage of waterlogging (T), the plant height (25.92 and 31.88 cm at 60 DAS and harvest respectively) was maximum with waterlogging at 50% flowering and it was followed by waterlogging at 25 DAE (days after emergence) and 15 DAE. Among the interaction effects, plant height at harvest (35.20) was maximum with 3 days of waterlogging from 50% flowering. The minimum plant height (14.19 cm) was recorded with 15 days of waterlogging from 15 DAE. The similar trend was observed at 60 DAS. Waterlogging alters the synthesis, metabolism and transport of endogenous hormones [13]. It results in the inhibition of IAA (auxin), gibberellic acid and cytokinin and increases the accumulation of abscisic acid and ethylene [14]. The reduction in plant height with increased waterlogging duration might be due to the inhibition of the growth promoting hormones [15, 16]. This is attributed to the increase in 1-amino cyclo propane-1-carboxylic acid (ACC), a precursor of ethylene synthesis and signaling molecule induced under hypoxic conditions [17]. It limits the shoot elongation and reduces photosynthesis exclusively at the earlier growth stage [18]. Similarly, Islam et al. [19] reported that five days waterlogging from 15 days after emergence reduced the plant height of mungbean by 28.57% due to the inhibition of growth promoting hormones. In addition to this, nitrogen is necessary for plants vegetative growth. Waterlogging reduces the soil available nitrogen due to denitrification and leaching. The reduced nitrogen availability in the early vegetative growth stage ultimately affects the plant growth. A study by Olorunwa et al. [20] reported that cowpea plant height was reduced due to lesser nutrient uptake attributed to restricted root growth with 10 days of waterlogging stress during vegetative growth stage.

### **3.1.2 Number of branches plant<sup>-1</sup>**

Across the growth stages, the number of branches at 60 DAS and harvest (3.40 and 3.53 plant<sup>-1</sup> respectively) was maximum with 3 days of waterlogging ( $D_1$ ) and minimum (2.37 and 2.41 plant<sup>-1</sup> respectively) with 15 days of waterlogging ( $D_7$ ). Among the growth stages, the number of branches was higher with waterlogging at 25 DAE and it was followed by 50% flowering and 15 DAE. Considering the interaction effects, the number of branches at harvest was the highest (4.00 plant<sup>-1</sup>) with 3 days of waterlogging from 25 DAE ( $D_1T_2$ ) and the lowest (2.00 plant<sup>-1</sup>) with 15 days of waterlogging from 15 DAE ( $D_7T_1$ ). Similar trend was noted at 60 DAS. The lesser number of branches

with waterlogging at early growth stage might be due to the increased energy spent in recovery mechanisms after relieving from waterlogging stress, whereas reduction in number of branches with increased duration of waterlogging can possibly be attributed to the related stress tolerance mechanisms which emphasize only prevention from stress rather than production of newer branches. Our findings are supported by Minchin et al. [21] who reported that waterlogging stressed cowpea plants during vegetative stage recorded the lowest number of branches ( $9.5 \text{ plant}^{-1}$ ). Similarly, 10 days waterlogging stress from 21 days after sowing recorded the lowest number of branches ( $4.20 \text{ plant}^{-1}$ ) in cowpea [7].

### **3.1.3 Leaf area**

Considering the waterlogging durations (D), the leaf area at harvest was highest ( $205.27 \text{ cm}^2 \text{ plant}^{-1}$ ) with waterlogging for 3 days ( $D_1$ ) and lowest with 15 days ( $D_7$ ) of waterlogging ( $49.14 \text{ cm}^2 \text{ plant}^{-1}$ ). However, the leaf area at 60 DAS was significantly unaffected by the durations of waterlogging. Among the stages of waterlogging (T), the leaf area at 60 DAS was highest ( $682.51 \text{ cm}^2 \text{ plant}^{-1}$ ) with waterlogging at 50% flowering. The leaf area at harvest was highest ( $149.68 \text{ cm}^2 \text{ plant}^{-1}$ ) with waterlogging at 25 DAE and it was on par with waterlogging at 15 DAE. The leaf area was lowest ( $68.60 \text{ cm}^2 \text{ plant}^{-1}$ ) with waterlogging from 50% flowering. Considering the interactions, leaf area at harvest was the highest ( $218.69 \text{ cm}^2 \text{ plant}^{-1}$ ) with 3 days of waterlogging from 25 DAE ( $D_1T_2$ ). Whereas, the lowest leaf area ( $0 \text{ cm}^2 \text{ plant}^{-1}$ ) was recorded with 15 days of waterlogging from 50% flowering ( $D_7T_3$ ). The similar trend was observed at 60 DAS. Extended period of waterlogging results in the inhibition of photosynthesis-related enzyme activities, decrease in the ability of leaf chlorophyll synthesis, which causes early senescence, yellowing, and peeling of the leaves and inhibits the growth of newer leaves [22]. This resulted in complete leaf fall at 13 and 15 days of waterlogging from 50% flowering. At harvest, the leaf area was decreased by 52.39% with waterlogging at 15 DAE, 44.83% with waterlogging at 25 DAE and 74.71% with waterlogging at 50% flowering. The reduction in leaf area was in the order of waterlogging at 50% flowering > 15 DAE > 25 DAE. From the interactions, it can be noted that the reduction in leaf area was higher with increased duration of waterlogging from 50% flowering. This was possibly due to the higher translocation of photosynthates to grain under stress conditions which ultimately cause earlier senescence of leaves than waterlogging during other growth stages. Our finding is in line with Ahmed et al. [23], who reported that waterlogging at reproductive stage of mungbean decreased the leaf area by 19.8 to 30.7%.

**Table 1. Effect of waterlogging on growth parameters of cowpea**

Treatment	Plant height (cm)		Number of branches plant <sup>-1</sup>		Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	
	60 DAS	At harvest	60 DAS	At harvest	60 DAS	At harvest
<b>Duration of waterlogging (D)</b>						
D <sub>1</sub> – 3 days	20.86 <sup>a†</sup>	25.07 <sup>a</sup>	3.40 <sup>a</sup>	3.53 <sup>a</sup>	615.66 <sup>a</sup>	205.27 <sup>a</sup>
D <sub>2</sub> – 5 days	19.99 <sup>ab</sup>	24.00 <sup>b</sup>	3.06 <sup>b</sup>	3.06 <sup>b</sup>	633.15 <sup>a</sup>	164.66 <sup>ab</sup>
D <sub>3</sub> – 7 days	19.00 <sup>bc</sup>	22.76 <sup>c</sup>	2.82 <sup>c</sup>	2.91 <sup>bc</sup>	626.87 <sup>a</sup>	136.56 <sup>bc</sup>
D <sub>4</sub> – 9 days	18.76 <sup>bc</sup>	21.71 <sup>d</sup>	2.76 <sup>cd</sup>	2.85 <sup>cd</sup>	565.03 <sup>a</sup>	102.27 <sup>cd</sup>
D <sub>5</sub> – 11 days	18.59 <sup>bc</sup>	21.24 <sup>d</sup>	2.62 <sup>cd</sup>	2.70 <sup>de</sup>	555.21 <sup>a</sup>	91.51 <sup>c-e</sup>
D <sub>6</sub> – 13 days	18.72 <sup>bc</sup>	20.26 <sup>e</sup>	2.57 <sup>d</sup>	2.63 <sup>e</sup>	557.41 <sup>a</sup>	63.03 <sup>de</sup>
D <sub>7</sub> – 15 days	18.38 <sup>c</sup>	19.7 <sup>f</sup>	2.37 <sup>e</sup>	2.41 <sup>f</sup>	556.00 <sup>a</sup>	49.14 <sup>e</sup>
<b>S.Em. ±</b>	0.30	0.232	0.070	0.067	34.81	17.02
<b>Time/stage of waterlogging (T)</b>						
T <sub>1</sub> – 15 DAE*	15.77 <sup>b</sup>	16.56 <sup>c</sup>	2.33 <sup>c</sup>	2.40 <sup>c</sup>	510.86 <sup>c</sup>	129.90 <sup>a</sup>
T <sub>2</sub> – 25 DAE	15.85 <sup>b</sup>	17.86 <sup>b</sup>	3.31 <sup>a</sup>	3.40 <sup>a</sup>	567.77 <sup>b</sup>	149.68 <sup>a</sup>
T <sub>3</sub> – 50% flowering	25.92 <sup>a</sup>	31.88 <sup>a</sup>	2.76 <sup>b</sup>	2.81 <sup>b</sup>	682.51 <sup>a</sup>	68.60 <sup>b</sup>
<b>S.Em. ±</b>	0.52	0.152	0.046	0.044	22.79	11.15
<b>Interaction (D×T)</b>						
<b>S.Em. ±</b>	1.37	0.402	0.122	0.116	60.30	29.49
<b>Control</b>	26.55	35.63	3.66	3.66	760.36	271.33
<b>S.Em. ±</b>	0.79	0.40	0.14	0.13	64.25	43.71
<b>CD at 5%</b>	2.28	1.14	0.41	0.39	183.37	124.75

\*DAE, days after emergence; †Means followed by the same letter (s) within the column are not significantly differed ( $P < 0.05$ ).

### 3.2 EFFECT OF WATERLOGGING ON PHYSIOLOGICAL PARAMETERS OF COWPEA

Waterlogging significantly affected the selected physiological parameters of cowpea at stress (Fig. 1a and b).

#### 3.2.1 Proline ( $\mu\text{mol g}^{-1}$ )

Among the waterlogging durations (D), the highest proline content ( $38.85 \mu\text{mol g}^{-1}$ ) was recorded with 11 days of waterlogging (D<sub>5</sub>) and remained steady up to 15 days of waterlogging (D<sub>7</sub>), whereas it was lowest ( $23.71 \mu\text{mol g}^{-1}$ ) at 3 days of waterlogging (D<sub>1</sub>). Among the stages, the proline content due to varied durations of waterlogging was in the order of 25 DAE > 15 DAE > 50% flowering. Considering the interaction effects, proline content was highest ( $43.70 \mu\text{mol g}^{-1}$ ) with 11 days of waterlogging from 25 DAE (D<sub>5</sub>T<sub>2</sub>). Proline accumulation under waterlogging stress is

considered an acclamatory mechanism [24]. It has the functional role of maintaining osmotic adjustment, stabilizing cellular structures and scavenge free radicals during stress [25]. The accumulation of proline with increased duration of waterlogging stress acts as an osmolyte and maintains the plant water status and hydraulic conductivity [26]. Similarly, in our study, the proline content was increased with higher intensity of waterlogging. The four times higher accumulation of proline due to waterlogging stress was earlier reported in groundnut [27], whereas, 8 days of waterlogging increased the proline content by 101 to 128% in pigeon pea [28].

### **3.2.2 Protein ( $\mu\text{g g}^{-1}$ )**

With regard to waterlogging durations (D), the leaf protein content was highest ( $31.48 \mu\text{g g}^{-1}$ ) with waterlogging for 3 days ( $D_1$ ), whereas it was lowest ( $20.48 \mu\text{g g}^{-1}$ ) with 15 days of waterlogging ( $D_7$ ). However, protein content was significantly unaffected by waterlogging during different growth stages (T). Among the interaction effects, protein content was the highest ( $31.92 \mu\text{g g}^{-1}$ ) with 3 days of waterlogging from 15 DAE ( $D_1T_1$ ) and lowest ( $19.7 \mu\text{g g}^{-1}$ ) with 15 days of waterlogging from 50% flowering ( $D_7T_3$ ). Protein content is highly susceptible to varying degrees of stress conditions [29]. Waterlogging stress causes the degradation of plant structural protein due to the dissociation of polyribosomes [30]. The restricted nitrogen uptake due to waterlogging reduces the protein content. Also, the accelerated anoxic metabolism restricts protein synthesis. Therefore, in our study, prolonged duration of waterlogging stress caused a significant decrease in plant protein content. The reduction in leaf protein content with high-intensity waterlogging was earlier reported in wheat [31], pigeon pea [32] and maize [33].

### **3.2.3 Normalized Difference Vegetation Index (NDVI)**

Among the durations of waterlogging (D), NDVI was highest (0.63) with 3 days ( $D_1$ ) and lowest with 15 days ( $D_7$ ) of waterlogging. With regard to the stages of waterlogging (T), NDVI values were observed in the trend of 25 DAE (0.61) > 50% flowering (0.52) > 15 DAE (0.49). Among the interactions, NDVI was highest (0.72) with 3 days of waterlogging from 25 DAE and 50% flowering ( $D_1T_2$  and  $D_1T_3$ ), whereas it was lowest (0.31) with 15 days of waterlogging from 50% flowering ( $D_7T_3$ ). NDVI indicates the health and greenness of plant. The increased waterlogging durations reduced the leaf chlorophyll content, subsequently caused yellowing, chlorosis, early senescence of

leaves and wilting. This is reflected in the reduced NDVI values with increased waterlogging intensity. The lower NDVI values with waterlogging from 15 DAE were due to lower crop canopy, whereas from 50% flowering it was due to higher senescence and leaf fall which resulted in reduced plant canopy. This is in line with **the findings** of Basavaraj et al. [7] who indicated that waterlogging stressed cowpea plants recorded lower NDVI values (0.38) compared to non-waterlogged plants (0.71).



**Fig. 1. Proline ( $\mu\text{mol g}^{-1}$ ), protein ( $\mu\text{g g}^{-1}$ ) content and NDVI of cowpea at stress as influenced by varied durations (a) and time/stages (b) of waterlogging.**

### **3.3 EFFECT OF WATERLOGGING ON YIELD ATTRIBUTES AND YIELD OF COWPEA**

The varied durations of waterlogging during different growth stages exhibited a considerable effect on the yield attributes and yield of cowpea (Table 2). Among the waterlogging durations (D), higher yield attributes viz., number of pods ( $4.27 \text{ plant}^{-1}$ ), pod length (15.24 cm) and number of seeds ( $14.27 \text{ pod}^{-1}$ ) and yield viz., grain ( $6.27 \text{ g plant}^{-1}$ ) and haulm yield ( $15.62 \text{ plant}^{-1}$ ) were recorded with 3 days of waterlogging ( $D_1$ ) and lower with 15 days of waterlogging ( $D_7$ ). The reduction in grain yield with increased duration of waterlogging can be attributed to higher flower abortion rate and poor pod setting [34, 8]. The stress during 50% flowering produced pods, they elongated and matured within short period. Hence there was less yield loss, though the plants were dried. Across the durations of waterlogging, waterlogging from 50% flowering recorded the higher yield attributes viz., number of pods ( $3.91 \text{ plant}^{-1}$ ), pod length (15.04 cm) and number of seeds ( $12.94 \text{ pod}^{-1}$ ) and yield viz., grain ( $5.14 \text{ g plant}^{-1}$ ) and haulm yield ( $14.79 \text{ plant}^{-1}$ ). The number of pods ( $2.94 \text{ plant}^{-1}$ ), number of seeds ( $12.94 \text{ pod}^{-1}$ ) and grain yield ( $3.03 \text{ g plant}^{-1}$ ) were lowest with waterlogging from 15 DAE. On contrary, pod length (13.70 cm) and haulm yield ( $10.71 \text{ g plant}^{-1}$ ) were lowest with waterlogging from

25 DAE. This indicates that though the plants with waterlogging from 15 DAE produced lengthier pods, it was unable to produce the higher number of seeds pod<sup>-1</sup> due to restriction in the source sink relationship which resulted in lesser biomass allocation for seeds leading to chaffy pods. Though the haulm yield was higher with waterlogging from 15 DAE, a lower grain yield was registered. This is linked to the lesser transport of metabolites and nutrients for better seed filling and remobilization of smaller amounts of pre-anthesis resources [35]. This may also be related to the higher energy spent on stress recovery than on reproductive growth [36, 37]. Among the interaction effects (D×T), the higher yield attributes and yield viz., grain (6.89 g plant<sup>-1</sup>) and haulm (19.73 g plant<sup>-1</sup>) yield were recorded with 3 days of waterlogging from 50% flowering (D<sub>1</sub>T<sub>3</sub>). However, the lower yield attributes and yield were recorded with 15 days of waterlogging from 15 DAE (D<sub>7</sub>T<sub>1</sub>). Though the pod length was lower (13.70 cm) with waterlogging from 25 DAE, the number of seeds pod<sup>-1</sup> was lesser with waterlogging from 15 DAE (12.94). The restriction in root growth reduces the grain yield of crop [38]. The increased duration of waterlogging especially at early growth stage hinders the root growth and development of roots resulting in reduced nutrient uptake, shoot growth and yield. Furthermore, the production of reactive oxygen species (ROS) due to waterlogging induces damage to the photosystem II activity resulting in reduced photosynthetic activity [39, 40].

**Table 2. Effect of waterlogging on yield attributes and yield of cowpea**

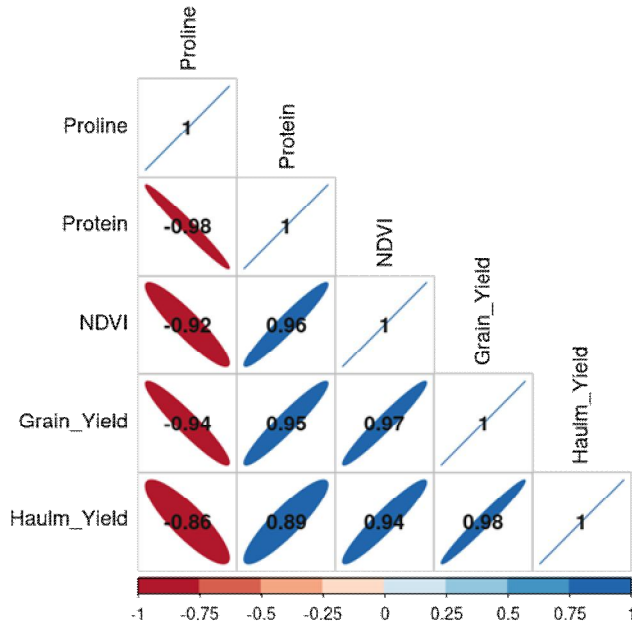
Treatment	Number of pods plant <sup>-1</sup>	Pod length (cm)	Number of seeds pod <sup>-1</sup>	Grain yield (g plant <sup>-1</sup> )	Haulm yield (g plant <sup>-1</sup> )
<b>Duration of waterlogging (D)</b>					
D <sub>1</sub> – 3 days	4.27 <sup>a†</sup>	15.24 <sup>a</sup>	14.27 <sup>a</sup>	6.27 <sup>a</sup>	15.62 <sup>a</sup>
D <sub>2</sub> – 5 days	4.01 <sup>ab</sup>	15.22 <sup>a</sup>	14.01 <sup>ab</sup>	5.57 <sup>b</sup>	14.96 <sup>a</sup>
D <sub>3</sub> – 7 days	3.68 <sup>b</sup>	15.00 <sup>ab</sup>	13.68 <sup>b</sup>	4.95 <sup>bc</sup>	13.90 <sup>b</sup>
D <sub>4</sub> – 9 days	3.73 <sup>b</sup>	14.82 <sup>b</sup>	13.73 <sup>b</sup>	4.29 <sup>c</sup>	13.05 <sup>c</sup>
D <sub>5</sub> – 11 days	3.23 <sup>c</sup>	14.48 <sup>c</sup>	13.23 <sup>c</sup>	3.14 <sup>d</sup>	11.18 <sup>d</sup>
D <sub>6</sub> – 13 days	3.10 <sup>cd</sup>	14.34 <sup>c</sup>	13.10 <sup>cd</sup>	2.53 <sup>de</sup>	8.92 <sup>e</sup>
D <sub>7</sub> – 15 days	2.73 <sup>d</sup>	14.16 <sup>c</sup>	12.73 <sup>d</sup>	2.40 <sup>e</sup>	8.21 <sup>f</sup>
<b>S.Em. ±</b>	0.14	0.264	0.14	0.23	0.24
<b>Time/stage of waterlogging (T)</b>					
T <sub>1</sub> – 15 DAE*	2.94 <sup>b</sup>	15.04 <sup>b</sup>	12.94 <sup>b</sup>	3.03 <sup>c</sup>	11.30 <sup>b</sup>
T <sub>2</sub> – 25 DAE	3.76 <sup>a</sup>	13.70 <sup>c</sup>	13.76 <sup>a</sup>	4.32 <sup>b</sup>	10.71 <sup>c</sup>
T <sub>3</sub> – 50% flowering	3.91 <sup>a</sup>	15.52 <sup>a</sup>	13.91 <sup>a</sup>	5.14 <sup>a</sup>	14.79 <sup>a</sup>
<b>S.Em. ±</b>	0.09	0.173	0.09	0.15	0.16
<b>Interaction (D×T)</b>					

<b>S.Em. ±</b>	0.25	0.457	0.25	0.40	0.42
<b>Control</b>	5.10	16.83	15.10	7.42	21.16
<b>S.Em. ±</b>	0.25	0.45	0.23	0.39	0.73
<b>CD at 5%</b>	0.71	1.29	0.71	1.12	2.09

\*DAE, days after emergence; †Means followed by the same letter (s) within the column are not significantly differed ( $P < 0.05$ ).

### 3.4 CORRELATION STUDIES BETWEEN PLANT PHYSIOLOGICAL PARAMETERS AND YIELD

The correlation studies between plant physiological parameters and yield was significantly affected by waterlogging stress (Fig. 2). The study indicates that the proline content had negative correlation with grain ( $r = -0.94$ ) and haulm ( $r = -0.86$ ) yield. This is contrary to the earlier findings that the increased proline content prevents the plants from stress [41, 42]. Whereas in this study, the proline content was increased up to 9 days of waterlogging and became steady after that, but the yield was decreased with increased duration of waterlogging. This resulted in the negative association. However, the protein content had positive correlation with grain ( $r = 0.95$ ) and haulm ( $r = 0.89$ ) yield. This result is consistent with previous studies that higher protein content is associated with higher nitrogen accumulation [43, 44]. Hence the maintenance of higher leaf protein content under waterlogging stress increases the yield during recovery. Similarly, the NDVI had positive correlation with grain ( $r = 0.97$ ) and haulm ( $r = 0.94$ ) yield. The higher NDVI values indicate better plant greenness and health linked with higher yield. This is in line with the findings of Basavaraj et al. [45].



**Fig. 2. Correlation coefficient between plant key physiological parameters and yield.**

#### 4. CONCLUSION

Cowpea cultivation during rainy season is severely affected by waterlogging due to unpredicted high-intensity rains. Hence, the assessment of cowpea to varied durations of waterlogging at different growth stages is **needed**. **The results** suggest that increased durations of waterlogging (3 to 15 days) during different growth stages (15 DAE, 25 DAE and 50% flowering) resulted in the drastic reduction in cowpea growth, and yield. Regardless of waterlogging durations, the lowest growth and yield were recorded with waterlogging at 15 DAE, followed by 25 DAE and 50% flowering. The reduction of key growth and associated physiological parameters reduced the grain and haulm yield. Moreover in this study, the physiological parameters; leaf protein content and NDVI had a positive correlation with yield, while the leaf proline content had negative relationship. From the current investigation, it was found that cowpea is sensitive to high-intensity waterlogging (beyond 3–5 days) especially during the early growth stage (15 DAE) over other stages. The findings of this research **serve as a** guide for **the** further studies on **planning mitigation** strategies to reduce the impact of high-intensity waterlogging during the sensitive growth stages **of cowpea**.

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1. Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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