PHYSIOLOGICAL AND BIOCHEMICAL STUDIES IN CLUSTER BEAN [*Cyamopsis tetragonoloba* (L.) Taub.] AS INFLUENCED BY LIGHT AND MOISTURE STRESS

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KERALA, INDIA

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By

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(2018-11-159)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT PHYSIOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM- 695 522
KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled **"PHYSIOLOGICAL AND BIOCHEMICAL STUDIES IN CLUSTER BEAN [*Cyamopsis tetragonoloba* (L.) Taub.] AS INFLUENCED BY LIGHT AND MOISTURE STRESS"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship or other similar title, of any other University or Society.

Place : Vellayani
Date : 18/09/2020

SREERAG
(2018-11-159)

CERTIFICATE

Certified that this thesis entitled **“PHYSIOLOGICAL AND BIOCHEMICAL STUDIES IN CLUSTER BEAN [*Cyamopsis tetragonoloba* (L.) Taub.] AS INFLUENCED BY LIGHT AND MOISTURE STRESS”** is a record of research work done independently by Mr. SREERAG under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Place : Vellayani
Date : 18/09/2020

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SREERAG

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LIST OF ABBREVIATIONS

CAT	:	Catalase
CD (0.05)	:	Critical difference at 5 % level
C_i	:	Intercellular carbon dioxide concentration
CGR	:	Crop growth rate
DAP	:	Days after planting
DAS	:	Days after sowing
DNA	:	Deoxyribo-nucleic acid
et al.	:	Co-workers/ Co-authors
Fig.	:	Figure
FR:R	:	Far red to Red ratio
g	:	Gram
g^{-1}	:	Per gram
G_s	:	Stomatal conductance
ha	:	Hectare
i.e	:	That is
Kg	:	Kilogram
LAI	:	Leaf area index
m^2	:	Square metre
mg	:	Milligram
mL	:	Millilitre
NS	:	Not significant
No.	:	Number
PBS	:	Phosphate buffered saline
P_n	:	Photosynthetic rate
POD	:	Peroxidase
PSM	:	Plant secondary metabolites

RGR	:	Relative growth rate
RBD	:	Randomized block design
SEm	:	Standard error of mean
SOD	:	Superoxide dismutase
t	:	Tonne
T_r	:	Transpiration rate
viz.,	:	Namely
WDS	:	Water deficit stress

LIST OF SYMBOLS

%	:	Per cent
@	:	at the rate of
°C	:	Degree Celsius
µ	:	Micro

Introduction

1. INTRODUCTION

Legumes have an indiscriminate position in Indian diets, the crops are rich in amylose starch. For diabetes patients, consuming lentils, peas, and beans controls blood sugar management. Legumes possess a lower glycemic index when compared with other carbohydrate sources. The Cluster bean or guar bean (*Cyamopsis tetragonoloba*) (L.) is an annual legume belonging to the family Leguminosae and subfamily Papilonaceae. The cultivation of cluster bean in India is known since ancient times for vegetable, manure and fodder purposes. It was most likely originated in Africa (Gillette 1958), which was domesticated primarily in Africa and Arabia making its way to the Indo-Pakistan subcontinent. India contributes to 80% of the world's production of cluster beans where it's grown during rainy season and the crop also tolerates arid conditions. Cultivation of cluster bean is best observed under full sunlight, well-drained soil, and frequent to moderate rainfall. The planting of crops is done after the first rains in July and harvested in late October. It is extensively grown in north-western India. Guar is also used as a cattle feed as well as green manure.

The Cluster-bean crop is known as 'kothamara or cheeniamara' in Malayalam and is an underexploited leguminous vegetable with high nutritional and medicinal properties, its deep tap roots enables the plant to reach moisture far below the soil surface. The Plants have single stems, fine branching, or basal branching (depending on the variety). The cluster bean plant produces white and pink flowers as well as green pods that contain the beans. The pods of cluster bean are used for vegetable purpose. The cluster bean, as it is more popularly known, is an annual crop, that is, it can be grown all over the year. It is a leguminous crop and a good source to take care of soil health. The crop can fix nearly 30 kg nitrogen/ha, the leaves of the crop adds organic carbon to the soil. Green pod vegetables are used for cooking purposes, whereas the mature varieties are used to make guar gum. Guar gum is utilized as a thickening agent, in food items like ice creams and pudding. They have a low-calorie value, and also have a huge number of medicinal benefits like the control of blood sugar levels, and reducing the cholesterol level of the body. The gum of cluster bean may stimulate a protective, pain-relieving and soothing effect on gastric ulcers, contribute to lowering cholesterol, blood pressure and blood sugar levels and also play a positive role in general weight loss (Sharma *et al*., 2011; James, 2002).

Stress is a constraint or highly unpredictable fluctuations imposed on regular metabolic patterns that cause injury, disease, or aberrant physiology caused by factors that tend to disrupt the equilibrium. Plants are frequently exposed to many stresses such as drought, low temperature, salt, flooding, heat, oxidative stress, and heavy metal toxicity, while growing in nature. Drought stress is considered to be a moderate loss of water, which leads to stomatal closure and limitation of gas exchange.

The seed extracts of *Cyamopsis tetragonoloba* L. indicate the presence of quinone, steroids, flavanoids, terpenoid, tannin, saponin, and phenol have been found. When subjected to light radiation, plants can adapt to the changes of circumstances by the release and accumulation of various secondary metabolites including phenolic compounds, triterpenoids and flavonoids, and many of them, have high economic and utilization value due to the prominent antioxidant property (Yang *et al*., 2018).

Within a living system a group of bioactive compounds called free radicals are produced during normal metabolism which have unpaired electrons that can initiate harmful chain reactions by targeting stable molecules, causing lipid peroxidation, damage of DNA, and proteins. Antioxidants compounds safeguard the cells against free radicals. Plants developed various antioxidant defence systems resulting in the formation of numerous potent antioxidant components also called as plant secondary metabolites (PSMs). PSMs like flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins, ββ--carotene and α-carotene and α--tocopherol are stated to be endowed with anti-oxidative property. Recently many studies have verified that water stress may possibly increase the amount of secondary metabolites in a wide variety of plant species. Hence light and moisture stress-induced physiological and biochemical studies may be of great significance in an industrially important crop such as Cluster bean. Hence the current study was carried out with the following objectives,

- To study the moisture stress-induced physiological and biochemical changes in Cluster bean (*Cyamopsis tetragonoloba*)
- To study the impact of artificial shade on physiological and biochemical changes

Review of literature

2. REVIEW OF LITERATURE

Plants are subjected to various kinds of environmental stresses during their growth and development. The normal metabolism of plants gets disturbed under stress conditions, which trigger a series of molecular, biochemical, physiological, and morphological changes and in turn lead to decreased growth and productivity. When exposed to stress conditions, the primary metabolisms of plants get affected due to unfavourable conditions created by different environmental factors. Plants in different stress conditions, try to reduce the effect of stress by accumulating more secondary metabolites**.**

Plant secondary metabolites are often denoted as compounds that have no vital role in the maintenance of life processes in plants, whereas they are important for the plants to interact with their environment for better adaptation and defence. Hence, understanding the imperative role played by them in plant growth and development is of great importance. Secondary metabolites have noteworthy practical applications in medicinal, nutritive and cosmetic purposes, in addition to their importance in adaptation of plants to numerous stresses. The production of these compounds is often low (less than 1% dry weight) and is influenced by the physiological and developmental stage of the plants (Akula and Ravishankar, 2011).

This review encompasses the research works related to various physiological and biochemical studies in cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.], as influenced by light and moisture stress. The cluster bean, a bushy annual herb has got a deep-root system and is a robust and drought-resilient leguminous crop grown in sandy soils of arid and semi-arid regions. It has been recognised as a priceless cash crop in the arid and semi-arid regions on account of its drought hardiness and a multitude of usage and has occupied a privileged place in the commercial sphere owing to its gum. The gum attained from cluster bean seeds is a choice of agrochemicals in paper, food, mining, cosmetics, textile, oil, and pharmaceutical industries across the world (Hymowitz and Matlock, 1963; Pawlik and Laskowski, 2006; NRAA, 2014).

Cluster bean seeds are used as a laxative and the boiled seeds are used to treat plague, inflammation, sprains (Khare, 2004), and arthritis (Katewa *et al.*, 2004). They also have anti-oxidant and anti-bilious properties and are used to treat liver enlargement. The methanolic extract of cluster bean seeds has anticancer activities (Badr *et al.*, 2014). It is also a popular choice in the manufacture of skin creams and gels to thicken and stabilize different preparations. Certainly, it is a natural and affordable plant-based alternative to be considered for various uses.

2.1 PHYSIOLOGICAL PARAMETERS

The changes within the surroundings result in transient changes in varied physiological features of plants. Plants react to the changes within the surroundings by modifying their physiology there by adapting and acclimating themselves to the new surroundings. Among the physiological parameters that are altered are photosynthetic rate, transpiration rate, crop growth rate, relative growth rate, chlorophyll content, etc.

2.1.1 EFFECT OF SHADE

When plants grow under either shade or high irradiance, the photosynthetic processes could be inhibited, simply because of the presence of too little or too much light which creates a stressful environment to the plants (Goncalves *et al*., 2005).

Guenni *et al.*, (2008) reported that in *Brachiaria sp.* three major physiological responses to shade have been observed including; a reduction in the respiration rate, an increase in the shoot to root partitioning of photosynthates, and an increase in the specific leaf area with a relatively low leaf mass ratio.

An increase in shoot dry weight with increasing shade levels has been found to negatively influence the harvest index. This influence is attributed to the increase in vegetative growth duration and decrease in root/shoot ratio under the shade, which allocate more assimilates for shoot rather than root growth (Sidique *et al*., 1990; Urbas and Zobel, 2000).

In response to shading, plants generally produce less dry matter, allocate more of their resources to shoot production, and have higher specific leaf areas that result in an overall increase in photosynthetic surface area (Grime, 1979). Also those plant species with morphological plasticity maintain comparably higher relative growth rates over a broad range of light intensities (Huxley 1967; Corre, 1983; Givnish, 1988).

In Sweet pepper cultivated under 40 and 60% of shading, a significant reduction in the net CO_2 assimilation rate, stomatal conductance, and transpiration rate were recorded. Also, the plants grown under 60% of shading were found to record higher chlorophyll 'a' and 'b' contents compared to those grown under 40% of shading (López *et al*., 2012). Cluster bean plants under shade transpire at a speedy rate in all the genotypes and consequently, the leaves maintain a lower temperature under shade (Vyas *et al*., 1996).

2.1.2 EFFECT OF MOISTURE STRESS

When water is withheld, processes such as transpiration, photosynthesis, leaf water relations, leaf expansion, flowering, or seed set remain unaffected, until a threshold soil water content is reached and at which point, these processes begin to decrease (Ray and Sinclair,1998; Liu and Stutzel,2002).

A reduction of photosynthetic rate due to drought stress has been reported in faba bean (Girma and Haile, 2014), grain legumes (Faroog *et al*., 2016) and common bean (Lanna *et al*., 2016). During drought, water deficit inside the plant tissue develops, leading to a significant inhibition of photosynthesis. In french bean, a reduction in photosynthetic rate due to stomatal closure has been reported (Sharkey and Seemann, 1989).

Pervez *et al*., (2009) analysed the impact of drought on tomato and in that study, stress was imposed at different growth stages of tomatoes. The four treatments included in the study were early stress, center anxiety, and delayed pressure, while for control, no stress was forced. In that study it was observed that a variety of expansion attributes *viz.* shoot extent, shoot fresh mass, root fresh heaviness, as well as plant height, decreased due to the obligation of the stress treatment, while in control the expansion attributes were improved.

Zeid and Shedeed (2006) worked on alfalfa (*Medicago sativa*) and reported that germination potential, hypocotyl length, shoot and root fresh and dry weight reduced due to water deficit stress, while the root length was found to improve under stress. Manikavelu *et al*. (2006) examined the consequence of drought on rice and stated that drought stress during the vegetative stage greatly reduced plant height and plant development.

When soil water is regularly exhausted, a certain amount of plant functions are affected and leaf expansion is one of the first to get affected. In barren mud, the growth of roots is greatly less depressed than expansion of shoot and there is characteristically a reduction in the desiccated mass percentage of shoot and root in response to drought stress (Hsiao, 2000).

2.2 BIOCHEMICAL PARAMETERS

Abiotic stress factors profoundly influence biochemical pathways responsible for the synthesis of organic compounds. This portion of the review encompasses the effects of light and moisture stress on the biochemical compounds.

2.2.1 MOISTURE STRESS AND METABOLISM

Water stress alters the cellular metabolism. Normally, under water stress, the rate of degradative processes is enhanced in contrast to synthetic reactions, for instance, protein, starch, etc. are reduced while sugars, amino acids, etc. are accumulated. Also during water stress, the redox potential gets shifted towards oxidised state and it causes an augmentation (higher concentration) of free radicles, reducing agents(akin to glutathione) and free radicle scavenging systems(such as superoxide dismutase) and this may neutralize the damage to plant cells. The sugars, amino acids, and betaines are regarded as protective solutes to different stresses.

Plants respond to stresses in many ways. They may escape the consequences of stress by completing their growth within the less stressful periods or they will suffer injury if the stress is present throughout and that they cannot cope up or else undergo specific changes in metabolism which help the plants either to avoid or tolerate the impact of stresses (Hopkins, 1995).

Water stress results in marked changes within the endogenous level of plant metabolites as well as amino acid, soluble sugar, and proteins (Joyce *et. al*., 1992), and some of these are postulated to boost drought resistance (Hanson and Hitz, 1982). Stress factors notably drought, destructively affect plant growth and development and cause a pointed decrease in plant productivity. The constraint in the availability of water induces osmotic stress (Molinari *et al*. 2007). In certain tolerant crop plants, morphological and metabolic changes occur in response to osmotic stress and contribute towards adaptation to such inevitable environmental constraints (Sinha *et al*., 1986).

Reports indicate that the total soluble protein content considerably improved under water deficit stress (WDS) in palmarosa (Fathima *et al*., 2002). But total leaf soluble protein was found to increase two and a half fold in *Vinca major* and nearly a twenty-fold in pumpkin once transferred from shade to open conditions (Logan *et al*., 1998).

Krishnamurthy *et al*., (2016) reported that tolerant genotypes of black pepper maintain higher root growth, higher relative water content, and lower cell membrane leakage under stress conditions. In general, tolerant genotypes amassed all the amino acids in higher quantities compared to susceptible ones during water stress. Photosynthetic rate, stomatal conductance, and transpiration rate were found to decrease with increasing water deficit.

The effect of drought was studied under both greenhouse and field conditions. Nearly all studies reported that sugars and organic acids increased in response to drought in most of the tomato accessions (Albert *et al.,* 2016a; Albert *et al*., 2016b) while others reported less strong effects (Wei *et al*., 2018).

2.2.2 LOW LIGHT STRESS AND METABOLISM

Total sugars, starch and nucleic acid content were reported to enhance in the shoot of various crop species like maize, rice, okra, french bean and groundnut grown under 20% shade(imposed from thirty days after sowing to maturity) (Singh, 1994). In a study with *Enicostemma littorale* plants, raised under short day (6 hour light), normal day and long day (24 hour light) conditions, the reducing sugar and total sugar content estimated from the leaves at monthly intervals were higher in long-day condition and lower in short-day condition (Vyas and Gajaria, 1998).

In another study with *Aloe vera,* a conventional medicinal plant grown under full sun, partial sun (30% full sun), and deep sun (10% full sun) for twelve to eighteen months, only minor treatmental effects were recorded in the concentration of soluble carbohydrate and aloin content in the leaf exudates. However, treatments with higher irradiance, failed to enhance the concentration of soluble carbohydrate and aloin content in the leaves. Huge limitations in irradiance also failed to induce them. Paez et al. (2000) reported that limitation in light availability primarily affect total dry mass production and allocation, without substantial effects on either primary or secondary carbon metabolites.

Shade is reported to augment the soluble sugars in *Ademnthera pavonina* (Krishnan and Rajendraprasad, 2000). Soluble protein and total organic nitrogen were found to accumulate markedly in the shoot of crop species *viz.*maize, rice, groundnut, and okra grown under low light stress (20% of shade) (Singh, 1994). Lee *et al.*, (1996) reported the effect of twenty days shade on protein, proline, and polyamines in aloe species. Shade diminished protein, proline, and polyamine in several species of aloe, whereas shade was found to increase total protein content in *Adenanthera pavonina* (Krishnan and Rajendraprasad, 2000).

The conditions of low irradiance and/or a high FR: R ratio causes a shift in carbon metabolism, toward the build-up of triacylglycerol, a storage lipoid in soybean leaves. Eighteen carbon fatty acid desaturation was additionally affected in highly shaded leaves whereas reduction in linolenic acid content was accompanied by a proportional increase in oleic and linolenic acid (Burkey *et al*., 1997).

In a study with tomato and chili pepper, it was reported that at 50% shade level there was a significant reduction in the number as well as weight of tomato fruits. Similar results were obtained in chili pepper also, along with lowered production of marketable fruits. However, 70% shade was reported to account for the highest reduction in the yield parameters of both tomato, chili and pepper. (Masabni *et al*., 2016)

Swertiamarin is a very important bitter glycoside of *Enicostemma littorale* and it is liable for the medicinal properties of the plant. Shade is reported to reduce the quantity of swertiamarin in *Enicostemma littorale* (Vyas, 2001). A study on growth, physiological and biochemical investigations in one year old 'YinHong' grape plants grown under different levels of shading (ranging from full sunlight (0% shade) to 80% reduction in sunlight) indicated that shading rate ≤45% did not significantly affect the growth of plants. But shading levels over 45%, inhibited the growth of grape plants. Also , soluble protein content, activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), chlorophyll content, net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO2 concentration (Ci) and transpiration rate (Tr) were found to decrease(Wu *et al*., 2018).

The number of leaf per plant decreased in Black pepper considerably under low light and leaves became thin under low light. An apparent decline in net assimilation rate (NAR) was observed with decrease in light intensity, which is expected as light is the driving force for photosynthesis and associated physiological process. In both the cultivars of black pepper decrease in transpiration was observed under shade. (Krishnaprasad *et al*., 2017).

Krishnaprasad *et al* ., (2017) studied the effect of shade on growth and physiological parameters in three varieties of tapioca. Net assimilation rate was severely reduced in all three varieties of cassava plants grown under shade. Since the amount of carbon fixed is low under shade, LAR is increased under shade. Similarly relative growth rate was reduced under shade. No significant differences in the partitioning between root and shoot was observed in Cassava. Photosynthetic rate was found to be significantly lower under shade. Transpiration rate depends on the stomatal conductance and all the three genotypes showed lower transpiration rate under shade compared to open condition.

Raai *et al.*, (2020) observed that shading generally showed most pronounced effect on the physiological traits of winged bean (*Psophocarpus tetragonolobus*) whereby the stomatal conductance, photosynthetic and transpiration rate differed significantly among the plants for all treatments. The non-shaded plants were observed to have superior growth and physiological responses than the shaded plants. It was also reported that the moderately shaded plants exhibited the highest yield per plant, which significantly differed from the non-shaded and heavily shaded plants.

A study conducted on the influence of shade regimes on photosynthetic rate and stomatal characters of ginger (*Zingiber officinale R.*) indicated that the highest photosynthetic rate was in plants grown under open condition, followed by plants grown at 20 to 40 per cent shade levels. Also it was found that the photosynthetic rate, stomatal conductance, transpiration rate, stomatal index and stomatal frequency significantly reduced in accordance with increasing levels of shade (Ajithkumar *et al*., 2002).

In a study on ginger with different shade levels *viz.* 20 and 40 per cent, it was reported that shade was found to be favourable for obtaining higher dry ginger yield.In that study,the volatile oil content was showing an increasing trend with increasing levels of shade and higher starch content was observed in plants grown under 20 percent shade level. Crude fibre content gradually reduced as the intensity of shade increased (Ajithkumar and Jayachandran, 2003).

In *Cucumus sativus* plants, free proline, soluble sugars, and total free amino acids decreased, with increase in shade levels. Concentration of free proline was found highest for shade 25% followed by 50% shade, 63%, shade and 75% shade. (Semida *et al*., 2017).

Materials and Methods

3. MATERIALS AND METHODS

The objective of the programme was to elicit information on the physiological and biochemical changes in cluster bean [*Cyamopsis tetragonoloba*(L.) Taub.] as influenced by light and moisture stress. In this regard, a field experiment was carried out at the Department of Plant Physiology, College of Agriculture, Vellayani during the year 2019-2020. In this experiment, cluster bean plants were raised in the field and the required treatments *viz.* light stress and moisture stress were imposed for a period of 90 days starting from two weeks after sowing. Physiological observations were taken and biochemical analysis were carried out at three different growth stages *viz.* vegetative stage (20-25 DAS), flowering stage (40-45 DAS) and pod filling stage (70 DAS) of the crop.

3.1. EXPERIMENTAL DETAILS

3.1.1 Location

A field experiment was conducted at the College of Agriculture, Vellayani, situated at $8^0$5'N latitude and $76^0$9'E longitude at an altitude of 29 m above mean sea level. The soil of the experimental field, used for the study, was redloam and belonged to the order Oxisol. The soil was acidic with a pH of 5.2 and with an EC of 0.002 dSm-1.

3.1.2 Season

The experiment was conducted from November 2019 to February 2020

3.1.3 Planting Material

Seeds of cluster bean [*Cyamopsis tetragonoloba*(L.) Taub.] variety Suruchi a variety released from KAU, were obtained from the Department of Vegetable Science, College of Agriculture, Vellayani.

3.1.4 Layout of the Experiment

The field experiment was laid out in factorial RBD design with nine treatments and four replications.

3.1.5 Outline of Technical Programme

The experiment was carried out in the field and the experiment consisted of nine different treatments that had combinations of moisture stress and light stress. The treatments included three levels each of moisture stress and shade. The water deficit stress levels given were 100 %, 75% and 50 % field capacities. The shade levels given were 50%, 25 % and open condition (control). Seeds were sown in the ridges in plots of 1.8x1.8 m with a spacing of 45x45 cm. Each plot accommodated 16 plants. Treatments were imposed for a period of 90 days starting from two weeks after sowing. The details of treatment combinations given are shown in Table 1.

Table 1. Details of shade and water deficit stress treatments imposed in cluster bean

Sl. No.	Treatment	Name of the treatment
1	T1	50% shade + 100 % Field capacity
2	T2	50% shade + 75 % Field capacity
3	T3	50% shade + 50 % Field capacity
4	T4	25% shade + 100 % Field capacity
5	T5	25% shade + 75 % Field capacity
6	T6	25% shade + 50 % Field capacity
7	T7	open + 100 % Field capacity
8	T8	open + 75 % Field capacity
9	T9	open + 50 % Field capacity

3.1.6 Preparation and Planting

The experiment was carried out in the field with 36 plots of 1.8x1.8 m size, each plot accommodating 16 plants. Before planting, farm yard manure (25 t/ha) was incorporated into the field as per the recommendations. Then lime (250 kg/hectare) was applied to neutralize the soil acidity. The experiment was laid out

Plate 1: Layout of the experimental field

Plate 2: General view of the experimental field

Plate 3: Vegetative stage of Cluster bean

Plate 4: Flowering stage of Cluster bean

Plate 5: Pod filling stage of Cluster bean

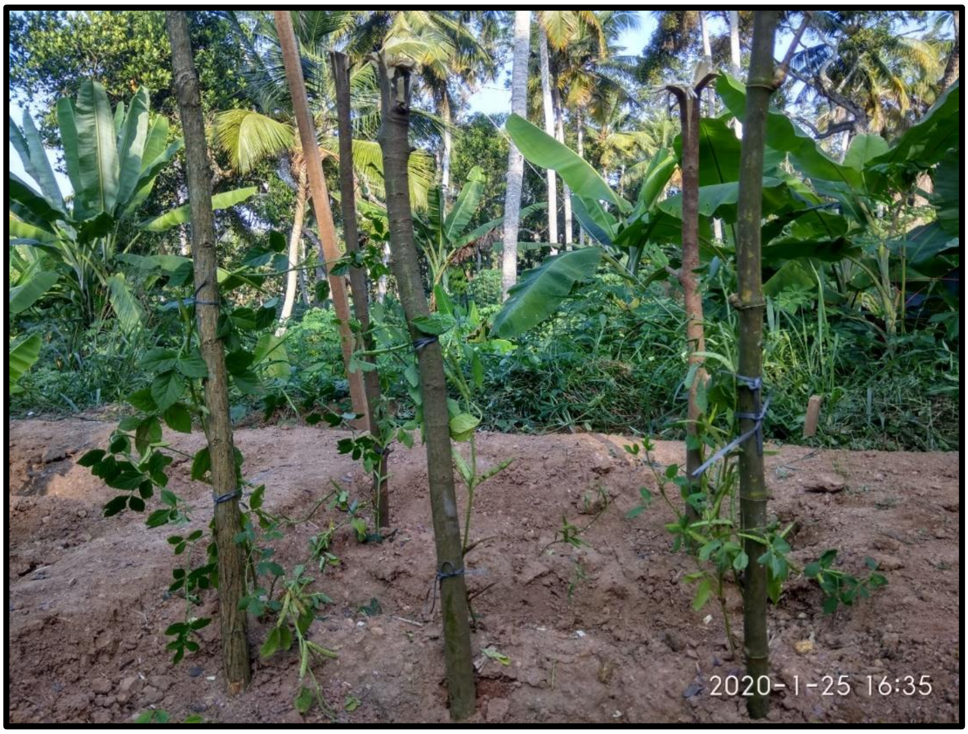

Plate 6: Cluster bean plants growing under open conditions

Plate 7: Cluster bean plant growing under 50% shade

Plate 8: Cluster bean plant growing under 25% shade

in factorial RBD design. The seeds were sown directly on the ridges. After 30 days of sowing, thinning was done and the healthy plants were retained. Observations for each parameters were recorded at vegetative stage (20-25 DAS), flowering stage (40-45 DAS) and pod filling stage (70 DAS).

3.1.7 Artificial shading/light stress

Two different shade levels (50 % and 25% shade) were supplied by making use of high-density polyethylene nets with differential light transmission, purchased from Kerala Agro Industries Corporation, which was spread over pandals at a height of 2 metres from the ground.

3.1.8 Moisture stress

Three irrigation regimes were followed to impose water stress of different field capacities which was determined by the method as given by Misra and Ahmed, (1987).

The field technique for finding the field capacity involved irrigating a test plot until the soil profile is saturated to a depth of about one meter. Then the plot was covered to prevent evaporation. The soil moisture was measured each 24 hours until the changes were found very less and the soil moisture content at that point was the estimate of field capacity and based on that different levels of field capacities were maintained by adjusting the irrigation schedule as mentioned below.

- Irrigation at an interval of 2 days – for 100% field capacity/(control)
- Irrigation at an interval of 4 days- for 75% field capacity
- Irrigation at an interval of 6 days- for 50% field capacity

3.2 OBSERVATIONS

3.2.1. Physiological characters

3.2.1.1Plant Height (cm)

Plant height was measured from the base of the plant to the apex of the plant, using a scale.

3.2.1.2 Primary branches per plant

The total number of primary branches developed per plant was counted and the mean value was expressed.

3.2.1.3 Root weight (g)

The plant was excavated for root studies in each replication and was separated into stem and root. The detached root was sun dried in envelopes for 3-4 days. Then, those samples were oven dried at 65℃ temperature till constant weight was reached, and then weighed with the help of electronic weighing balance.

3.2.1.4 Shoot weight (g).

The stem was detached from the excavated plant and was sun dried in envelopes for 3-4 days. Then, the samples were oven dried at 65℃ temperature till constant weight was reached and then weighed with the help of electronic weighing balance.

3.2.1.5 Root: shoot ratio

It was calculated by dividing root dry weight by the shoot dry weight of the plants from each replication.

3.2.1.6 Crop growth rate (mg cm^{-2} day^{-1})

$$CGR=\frac{(Tw_2-Tw_1)\text{x } 1000}{\text{land area x}(T_2-T_1)}$$

Where,

Tw_1 and Tw_2: were total dry weight plant^{-1} at 1st stage and 2nd stage (including root weight) at time t_1 and t_2 respectively

$T_2 - T_1$: was time interval between the two stages of recording total dry weight in days

3.2.1.7 Relative growth rate (mg g^{-1} day^{-1})

$$RGR=\frac{(Tw_2-Tw_1)\text{x } 1000}{(T_2-T_1)\text{ x } Tw_1}$$

Where,

Tw_1 and Tw_2: were total dry weight plant^{-1} at 1st stage and 2nd stage (including root weight) at time t_1 and t_2 respectively

$T_2 - T_1$: was time interval between the two stages of recording total dry weight in days

3.2.1.8 Photosynthetic rate

It was measured directly by using LCA-4 (Leaf Chamber Analyser or portable CO_2 analyser) manufactured by Analytical Development Co. Ltd, UK. The values were expressed in µ mol CO_2 $m^{-2}s^{-1}$

3.2.1.9 Transpiration rate

Transpiration rate was measured directly by using the portable CO_2 analyser or leaf chamber analyser (LCA-4), manufactured by Analytical Development Co. UK. The values were expressed in m mol H_2O $m^{-2}s^{-1}$.

3.2.2 Biochemical characters

3.2.2.1 Estimation of Chlorophyll content (DMSO method)

A weighed quantity of leaf sample (0.5g) was taken and cut into small bits. Those bits were put in test tubes and incubated overnight at room temperature, after pouring 10 ml DMSO: 80% acetone mixture (1:1 v/v). The coloured solution was decanted into a measuring cylinder and made up to 25 ml with the DMSO-acetone mixture. The absorbance was measured at 663 and 645 nm using a spectrophotometer. The chlorophyll content was obtained by substituting the absorbance values in the given formula.

Chl a = (12.7 x A_{663}-2.69 x A_{645}) x V/1000 x 1/ Fresh weight

Chl b = (22.9 x A_{645}-4.68x A_{663}) x V/1000 x 1/ Fresh weight

Total Chl (a + b) = (8.02 x A_{663}+ 20.2 x A_{645}) x V/1000 x 1/ Fresh weight

3.2.2.2 Estimation of Total Soluble Protein (Bradford method)

The total soluble protein was estimated following the Bradford method (1976). Leaf sample (0.5 g) was homogenised in 5 mL of PBS. A series of protein samples were prepared in PBS. The experimental samples were prepared in 10 microliter of PBS. A known volume (5 ml) of diluted dye binding solution was added to each tube. The solution was mixed well and allowed to develop a blue colour for at least 5 min. but no longer than 30 min. The red dye turned to blue when it bound to protein and its absorbance was measured at 596 nm. A standard curve was plotted using the field plot absorbance versus concentration. The protein in the experimental sample was calculated using the standard curve.

Results

3. RESULTS

The current investigation was carried out to study the light and moisture stress induced physiological and biochemical changes in cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]. The plants were subjected to a combination of moisture stress and low light stress for the study. Three different levels of moisture studied were 100%, 75% and 50% field capacities and the three levels of light stress studied were 50% shade, 25% shade and open full sunlight condition. The growth parameters, physiological and biochemical parameters were recorded at three growth stages to study the changes occurring in the plants as a result of light and moisture stresses. The results of the experiment carried out to address the above mentioned objective is given below.

4.1 PHYSIOLOGICAL PARAMETERS

4.1.1 Plant Height

The effect of different shade levels and field capacities on plant height of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 2. Among the treatments, a significant variation in plant height was observed between the treatments. The plants grown under shade were taller compared to the plants grown in open condition. The plants were tolerant up to 50% shaded condition. Of all the treatments, T6 (25% shade + 50% field capacity) recorded a maximum height of 53.75 cm, 83.75 cm and 133.75 cm in vegetative, flowering and pod filling stages respectively, which was to an extent of 9.38% in comparison to the control, T7 (open + 100% field capacity). Plants kept in open condition showed reduction in height compared to the shade grown plants. A significant reduction in height of the plants was observed in treatment T2 (50% shade + 75% field capacity) and T9 (open + 50% field capacity) at all the three growth stages studied (34.75 cm, 64.75 cm and 114.75 cm; 35 cm, 65 cm and 115 cm respectively). In terms of percentage the plant height of T2 and T9 were reduced by 13.61% and 13.31% respectively in comparison to the control, T7. It was observed that the treatments, T4 (25% shade

+ 100% field capacity), T5 (25% shade + 75% field capacity); T1 (50% shade + 100% field capacity) and T7, T3 (50% shade + 50% field capacity) with T9 and T2; T9 and T2 were statistically on-par with each other across all the growth stages studied. Among the shade levels 25% shade (S2) accounted for the highest value of plant height in all the growth stages studied, which enhanced the plant height by 14.1% in comparison to the control level, open (S3). Among moisture stress levels, 100% field capacity (F1) resulted in highest values for plant height in all the growth stages studied.

Table 2. Effect of different shade levels and moisture levels on plant height (cm) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	46.50	76.50	126.50	83.17
T2=S1F2	34.75	64.75	114.75	71.42
T3=S1F3	36.00	66.00	116.00	72.67
T4=S2F1	51.75	81.75	131.75	88.42
T5=S2F2	51.50	81.50	131.50	88.17
T6=S2F3	53.75	83.75	133.75	90.42
T7=S3F1	46.00	76.00	126.00	82.67
T8=S3F2	43.00	73.00	123.00	79.67
T9=S3F3	35.00	65.00	115.00	71.67
SE(m)	0.591	0.591	0.591	
CD (0.05)	1.734	1.734	1.734	
S1	39.083	69.083	119.083	75.750
S2	52.333	82.333	132.333	89.000
S3	41.333	71.333	121.333	78.000
SE(m)	0.341	0.341	0.341	
CD (0.05)	1.001	1.001	1.001	
F1	48.083	78.083	128.083	84.750
F2	43.083	73.083	123.083	79.750
F3	41.583	71.583	121.583	78.250
SE(m)	0.341	0.341	0.341	
CD (0.05)	1.001	1.001	1.001	

4.1.2 Number of primary branches per plant

The effect of different shade levels and field capacities on number of primary branches per plant of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 3. The treatments did not show significant variation in the number of primary branches per plant except for flowering stage. However, in the flowering stage, most of the treatment means were statistically insignificant, except for the control, T7 (open + 100% field capacity), which was significantly different from all other treatment means. The highest number of primary branches per plant were found in the control, T7 (open + 100% field capacity) i.e. 6.5, 8.5 and 10 for vegetative, flowering and pod filling stages respectively. The number of primary branches were highest in the plants grown in the open compared to shade. The number of primary branches were found to decrease with increasing water deficit within a particular shade level. A significant reduction in the number of primary branches per plant was observed for treatment T3 (50% shade + 50% field capacity) at all the growth stages studied (3.25, 4.5 and 6 respectively), to a degree of 45% in comparison to the control, T7. It was noticed that in the flowering stage, T8(open + 75% field capacity) was statistically on-par with T4(25% shade + 100% field capacity) and T9(open + 50% field capacity); T4 was on-par with T9, T6(25% shade + 100% field capacity), T1 (50% shade + 100% field capacity), T2(50% shade + 75% field capacity) and T3; T9 was on-par with T6, T1, T2 and T5(25% shade + 75% field capacity); T6 was on-par with T1, T2, T5 and T3; T1 was on-par with T2, T5 and T3; whereas T2 was on-par with T5 and T3. Among the shade levels open condition (S3) accounted for the highest value of number of primary branches in all the growth stages studied. It was also observed that the shade levels, S2 (25% shade) and S1 (50% shade) were statistically on-par in the flowering and pod filling stages. Among moisture stress levels, 100% field capacity (F1) resulted in highest values for number of primary branches per plant in all the growth stages studied. It was noticed that the impact of field capacity on the number of primary branches per plant was statistically insignificant for the pod filling stage of cluster bean. In the vegetative and flowering stages, F2 (75% field capacity) and F3 (50% field capacity) were found to be statistically on-par.

Table 3. Effect of different shade levels and moisture levels on number of primary branches per plant of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	3.75	5.25	7.00	5.33
T2=S1F2	3.50	5.25	6.75	5.17
T3=S1F3	3.25	4.50	6.00	4.58
T4=S2F1	4.50	6.00	7.25	5.92
T5=S2F2	4.00	5.00	6.75	5.25
T6=S2F3	4.00	5.50	7.25	5.58
T7=S3F1	6.50	8.50	10.00	8.33
T8=S3F2	5.50	6.75	9.75	7.33
T9=S3F3	4.75	6.00	9.25	6.67
SE(m)	0.332	0.348	0.377	
CD (0.05)	NS	1.021	NS	
S1	3.500	5.000	6.583	5.028
S2	4.167	5.500	7.083	5.583
S3	5.583	7.083	9.667	7.444
SE(m)	0.192	0.201	0.217	
CD (0.05)	0.564	0.590	0.638	
F1	4.917	6.583	8.083	6.528
F2	4.333	5.667	7.750	5.917
F3	4.000	5.333	7.500	5.611
SE(m)	0.192	0.201	0.217	
CD (0.05)	0.564	0.590	NS	

4.1.3 Root weight

The effect of different shade levels and field capacities on root weight of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 4. Among the treatments, a significant variation in root weight was observed between the treatments, except that of pod filling stage. The root weight of plants was higher under shade compared to the plants grown under open condition. The plants were tolerant up to 50% shaded condition as well as performed better under 50% field capacity. It was observed that of all the treatments, T3 (50% shade + 50% field capacity) recorded a maximum root dry weight of 0.47 g, 0.63 g and 0.72 g in vegetative, flowering and pod filling stages respectively, to a degree of 41.09% in comparison to the control, T7 (open + 100% field capacity). It was observed that in the vegetative stage, the treatments T1 (50% shade + 100% field capacity) and T6 (25% shade + 50% field capacity); T5 (25% shade + 75% field capacity) and T7; T7 and T4 (25% shade + 100% field capacity) were statistically on-par. In the flowering stage, T2 (50% shade + 75% field capacity) was on-par with T1 and T4 (25% shade + 100% field capacity); T1 was on-par with T4 and T5; T4 and T5 were on-par; whereas the treatment means, T8 (open + 75% field capacity) and T7 were statistically similar. The root weight was found to increase with increasing water deficit except for the plants grown under open condition in the vegetative stage. A significant reduction in root weight was observed in treatment T9 i.e. 0.26 g in the vegetative stage, the reduction amounting to 18.75% from the control, T7. However, in contrast, the control (T7) plants were found to have the lowest values of root weight under flowering and pod filling stages (0.44 g and 0.53 g respectively). Among the shade levels 50% shade (S1) accounted for the highest value of root weight in all the growth stages studied, promoting the root dry weight by 29.16% and among moisture stress levels, 50% field capacity (F3) resulted in highest values for root weight, furthering the root dry weight by 10.46% in all the growth stages under study.

Table 4. Effect of different shade levels and moisture levels on root weight (g) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	0.39	0.54	0.62	0.52
T2=S1F2	0.43	0.55	0.66	0.55
T3=S1F3	0.47	0.63	0.72	0.61
T4=S2F1	0.31	0.53	0.63	0.49
T5=S2F2	0.34	0.52	0.64	0.50
T6=S2F3	0.38	0.58	0.67	0.54
T7=S3F1	0.32	0.44	0.53	0.43
T8=S3F2	0.29	0.46	0.53	0.43
T9=S3F3	0.26	0.49	0.57	0.44
SE(m)	0.009	0.009	0.010	
CD (0.05)	0.026	0.027	NS	
S1	0.430	0.572	0.668	0.557
S2	0.341	0.544	0.646	0.510
S3	0.288	0.462	0.543	0.431
SE(m)	0.005	0.005	0.006	
CD (0.05)	0.015	0.016	0.017	
F1	0.341	0.501	0.593	0.478
F2	0.352	0.508	0.609	0.490
F3	0.367	0.568	0.655	0.530
SE(m)	0.005	0.005	0.006	
CD (0.05)	0.015	0.016	0.017	

4.1.4. Shoot weight

Among the treatments, a significant variation in shoot weight was observed across all the critical growth stages. The effect of different shade levels and field capacity levels on shoot weight of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 5. The shoot weight of plants grown under shade was lower compared to those plants grown under open condition. Of all the treatments, T7 (open + 100% field capacity) recorded the maximum shoot dry weight of 1.44 g, 2.65 g and 3.63 g in vegetative, flowering and pod filling stages respectively. A significant reduction in shoot weight of plants was observed in treatment T3(50% shade + 50% field capacity) i.e. 0.71 g and 1.76 g, diminishing the shoot dry weight by 50.69% and 33.58% for vegetative and flowering stages respectively, compared to the control, T7. Treatment T2 (50% shade + 75% field capacity) accounted for the lowest value in pod filling stage, where the reduction in the shoot dry weight amounted to 42.15%. The shoot dry weight was found to decrease with the increasing water deficit among the different treatments. It was observed that in the vegetative stage, T6 (25% shade + 50% field capacity) was statistically on-par with T1 (50% shade + 100% field capacity) and T2; T1 was on-par with T2; and T2 was on-par with T3. In the flowering stage, T6 was statistically on-par with T1; T1 was on-par with T2; whereas T2 was statistically similar to T3. In pod filling stage, T5 was statistically on-par with T6; T6 was on-par with T3; whereas T3 was on-par with T1.Among the shade levels open condition (S3) accounted for the highest value of shoot weight in all the growth stages studied and among moisture stress levels, 100% field capacity (F1) resulted in highest values for shoot weight. It was observed that F2 (75% field capacity) and F3 (50% field capacity) were statistically on-par in the pod filling stage.

Table 5. Effect of different shade levels and moisture levels on shoot dry weight (g) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	0.76	1.82	2.14	1.57
T2=S1F2	0.74	1.79	2.10	1.54
T3=S1F3	0.71	1.76	2.17	1.55
T4=S2F1	0.92	2.01	2.26	1.73
T5=S2F2	0.82	1.92	2.22	1.65
T6=S2F3	0.76	1.86	2.19	1.60
T7=S3F1	1.44	2.65	3.63	2.57
T8=S3F2	1.39	2.43	3.43	2.42
T9=S3F3	1.32	2.34	3.35	2.34
SE(m)	0.016	0.014	0.013	
CD (0.05)	0.046	0.041	0.038	
S1	0.738	1.789	2.135	1.554
S2	0.830	1.931	2.221	1.661
S3	1.383	2.471	3.469	2.441
SE(m)	0.009	0.008	0.007	
CD (0.05)	0.027	0.024	0.022	
F1	1.038	2.159	2.673	1.957
F2	0.983	2.045	2.581	1.870
F3	0.931	1.987	2.571	1.830
SE(m)	0.009	0.008	0.007	
CD (0.05)	0.027	0.024	0.022	

4.1.5 Root: shoot ratio

The effect of different shade levels and field capacity levels on the root: shoot ratio of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 6 . A significant variation in the root: shoot ratio was observed among the treatments for only the vegetative stage. A general increase in the root: shoot ratio was observed for the plants kept under artificial shades compared to the plants under open conditions. Among the treatments, T3 (50% shade + 50% field capacity) recorded the maximum root: shoot ratio (0.66, 0.36 and 0.33) for vegetative, flowering and pod filling stages respectively, to a magnitude of 154.72%, compared to the control, T7 (open + 100% field capacity). A significant reduction in the root: shoot ratio i.e. 0.20, was observed in treatment T9 (open + 50% field capacity) at the vegetative stage. This reduction accounted for a decrease of 9.09% in comparison to the control, T7. In the vegetative stage, T1 (50% shade + 50% field capacity) was statistically on-par with T6 (25% shade + 50% field capacity); T7 was on-par with T8 (open + 75% field capacity) and T9; whereas T8 was on-par with T9. Among the shade levels, 50% shade (S1) accounted for the highest value of root: shoot ratio across all the growth stages, resulting in an increase of 54.68% in comparison to the control level, open (S3). Among moisture stress levels, 50% field capacity (F3) resulted in the highest values for the root: shoot ratio in all the growth stages studied, amounting to an increase of 20.69% in comparison to control level, F1 (100% field capacity).

Table 6. Effect of different shade levels and moisture levels on the root: shoot ratio of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	0.52	0.30	0.29	0.370
T2=S1F2	0.58	0.30	0.32	0.400
T3=S1F3	0.66	0.36	0.33	0.450
T4=S2F1	0.35	0.27	0.28	0.300
T5=S2F2	0.41	0.27	0.29	0.320
T6=S2F3	0.50	0.31	0.31	0.373
T7=S3F1	0.22	0.17	0.14	0.180
T8=S3F2	0.21	0.19	0.16	0.187
T9=S3F3	0.20	0.21	0.17	0.193
SE(m)	0.015	0.006	0.004	
CD (0.05)	0.043	NS	NS	
S1	0.584	0.321	0.313	0.406
S2	0.418	0.283	0.293	0.331
S3	0.209	0.188	0.155	0.184
SE(m)	0.008	0.003	0.002	
CD (0.05)	0.025	0.010	0.006	
F1	0.361	0.242	0.238	0.280
F2	0.399	0.256	0.253	0.303
F3	0.451	0.294	0.270	0.338
SE(m)	0.008	0.003	0.002	
CD (0.05)	0.025	0.010	0.006	

4.1.6 Crop growth rate

A significant variation in the crop growth rate was observed among the treatments across all the growth stages. The effect of different shade levels and field capacities on the crop growth rate of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 7 . The crop growth rate was found to increase up to the flowering stage and then it declined at the pod filling stage. However, in the plants kept under open conditions there was a steady increase in the CGR, up to the pod filling stage. A general increase in the crop growth rate was recorded in the plants kept under open conditions, compared to the plants under shade. Among the treatments, the control T7 (open + 100% field capacity) recorded the maximum crop growth rate (0.48 mg cm^{-2} day^{-1}, 0.82 mg cm^{-2} day^{-1} and 0.94 mg cm^{-2} day^{-1}) at vegetative, flowering and pod filling stages respectively. A significant reduction in the crop growth rate was observed in the treatment T3 (50% shade + 50% field capacity) in all the three growth stages studied (0.31 mg cm^{-2} day^{-1}, 0.53 mg cm^{-2} day^{-1} and 0.22 mg cm^{-2} day^{-1} respectively), which accounted for a reduction of 52.68% , compared to the control, T7. It was noticed that in the vegetative stage, T4(25% shade + 100% field capacity) was statistically on-par with T8(open + 75% field capacity) and T9(open + 50% field capacity); T1(50% shade + 100% field capacity) was statistically on-par with T2(50% shade + 75% field capacity), T3, T5(25% shade+ 75% field capacity) and T6(25% shade+ 50% field capacity). In the flowering stage, T9 was statistically on-par with T8; whereas T5 was on-par with T6. In the pod filling stage, T8 was statistically on-par with T9; T4 with T5 and T6; whereas T1 was statistically on-par with T2 and T3. Among the shade levels, open condition (S3) accounted for the highest value of crop growth rate in all the growth stages studied. Among moisture stress levels, 100% field capacity (F1) resulted in highest values for the crop growth rate in all the growth stages studied.F2 (75% field capacity) and F3 (50% field capacity) were statistically on-par in the vegetative stage.

Table 7. Effect of different shade levels and moisture levels on crop growth rate (mg cm^{-2} day^{-1}) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	0.32	0.62	0.24	0.39
T2=S1F2	0.32	0.57	0.23	0.37
T3=S1F3	0.31	0.53	0.22	0.35
T4=S2F1	0.35	0.73	0.27	0.45
T5=S2F2	0.32	0.67	0.27	0.42
T6=S2F3	0.32	0.67	0.26	0.42
T7=S3F1	0.48	0.82	0.94	0.75
T8=S3F2	0.35	0.77	0.91	0.68
T9=S3F3	0.35	0.78	0.90	0.68
SE(m)	0.005	0.006	0.004	
CD (0.05)	0.014	0.017	0.012	
S1	0.315	0.571	0.228	0.371
S2	0.329	0.691	0.268	0.429
S3	0.438	0.781	0.918	0.712
SE(m)	0.003	0.003	0.002	
CD (0.05)	0.008	0.010	0.007	
F1	0.383	0.720	0.485	0.529
F2	0.353	0.670	0.469	0.497
F3	0.346	0.653	0.459	0.486
SE(m)	0.003	0.003	0.002	
CD (0.05)	0.008	0.010	0.007	

4.1.7 Relative growth rate

The effect of different shade levels and field capacity levels on the relative growth rate of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 8 . A significant variation in the relative growth rate was observed among the treatments across all the growth stages. The relative growth rate showed a decreasing trend throughout all the growth stages with the decline most evident at the pod filling stage. A general increase in the relative growth rate was recorded in the plants kept under open conditions, compared to the plants under shade. Among the treatments, the control T7 (open + 100% field capacity) recorded the maximum relative growth rate (100.225 mg g^{-1} day^{-1}, 69.225 mg g^{-1} day^{-1} and 32.2 mg g^{-1} day^{-1}) at vegetative, flowering and pod filling stages respectively. A significant reduction in the relative growth rate was observed in the treatment T3 (50% shade + 50% field capacity) in all the three growth stages studied (65.325 mg g^{-1} day^{-1}, 41.1 mg g^{-1} day^{-1} and 17.4 mg g^{-1} day^{-1} respectively). The decline in the RGR amounted to 38.59%, compared to the control, T7. In the vegetative stage, T8 (open + 75% field capacity) was found to be statistically on-par with T9 (open + 50% field capacity); T6 (25% shade + 50% field capacity) was on-par with T1 (50% shade + 100% field capacity) and T2 (50% shade + 75% field capacity); whereas, T1 was on-par with T2. In the flowering stage, T8 and T9 were statistically on-par treatments, whereas in the pod filling stage T1 and T2 were on-par. Among the shade levels, open condition (S3) accounted for the highest value of relative growth rate in all the growth stages studied. Among moisture stress levels, 100% field capacity (F1) resulted in highest values for the relative growth rate in all the growth stages studied. It was noticed that the moisture stress levels, F2 (75% field capacity) and F3 (50% field capacity) were statistically on-par for the flowering and pod filling stages

Table 8. Effect of different shade levels and moisture levels on relative growth rate (mg g^{-1} day^{-1}) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	70.325	45.475	18.350	44.72
T2=S1F2	70.250	43.175	18.250	43.89
T3=S1F3	65.325	41.100	17.400	41.28
T4=S2F1	73.350	53.050	25.200	50.53
T5=S2F2	71.450	51.125	22.425	48.33
T6=S2F3	70.375	50.550	21.325	47.42
T7=S3F1	100.225	69.225	32.200	67.22
T8=S3F2	97.275	60.175	28.625	62.03
T9=S3F3	96.325	59.525	28.250	61.37
SE(m)	0.086	0.079	0.061	
CD (0.05)	0.252	0.232	0.180	
S1	68.633	43.250	18.000	43.292
S2	71.725	51.575	22.983	48.761
S3	97.942	62.975	29.692	63.536
SE(m)	0.050	0.046	0.035	
CD (0.05)	0.146	0.134	0.014	
F1	81.300	55.917	25.250	54.156
F2	79.658	51.492	23.100	51.417
F3	77.342	50.392	22.325	50.020
SE(m)	0.050	0.046	0.035	
CD (0.05)	0.146	0.134	0.104	

4.1.8 Photosynthetic rate

The effect of different shade levels and field capacity levels on the photosynthetic rate of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 9. A significant variation in the photosynthetic rate was observed among the treatments across all the growth stages. The photosynthetic rate increased up to the flowering stage and then declined at the pod filling stage. A general increase in the photosynthetic rate was recorded in the plants kept under open conditions, compared to the plants under shade. Among the treatments, the control T7 (open + 100% field capacity) recorded the maximum photosynthetic rate (10.35 µ mol CO_2 m^2 s^{-1}, 14.65 µ mol CO_2 m^2 s^{-1} and 9.4 µ mol CO_2 m^2 s^{-1}) at vegetative, flowering and pod filling stages respectively. A significant reduction in the relative growth rate was observed in the treatment T3 (50% shade + 50% field capacity) in all the three growth stages studied (5.2 µ mol CO_2 m^2 s^{-1}, 5.7 µ mol CO_2 m^2 s^{-1} and 3.35 µ mol CO_2 m^2 s^{-1} respectively), whereby the photosynthetic rate was reduced by 58.58% in comparison to the control, T7. It was observed that in the pod filling stage, T7 and T8 (open + 75% field capacity) were statistically on-par; T6 (25% shade + 50% field capacity) was on-par with T5 (25% shade + 75% field capacity); whereas T1 (50% shade + 100% field capacity) was statistically on-par with T2 (50% shade + 75% field capacity). Among the shade levels, open condition (S3) accounted for the highest value of photosynthetic rate in all the growth stages studied. Among moisture stress levels, 100% field capacity (F1) resulted in highest values for the photosynthetic rate in all the growth stages studied.

Table 9. Effect of different shade levels and moisture levels on photosynthetic rate (µ mol CO_2 m^{-2} s^{-1}) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	6.100	6.325	4.325	5.58
T2=S1F2	5.750	6.100	4.250	5.37
T3=S1F3	5.200	5.700	3.350	4.75
T4=S2F1	7.700	9.550	5.600	7.62
T5=S2F2	7.200	8.200	5.175	6.86
T6=S2F3	6.850	7.775	5.225	6.62
T7=S3F1	10.350	14.650	9.400	11.47
T8=S3F2	9.625	13.650	9.250	10.84
T9=S3F3	9.100	13.425	8.950	10.49
SE(m)	0.048	0.053	0.071	
CD (0.05)	0.142	0.156	0.209	
S1	5.683	6.042	3.975	5.233
S2	7.250	8.508	5.333	7.030
S3	9.692	13.908	9.200	10.933
SE(m)	0.028	0.031	0.041	
CD (0.05)	0.082	0.090	0.121	
F1	8.05	10.175	6.442	8.222
F2	7.525	9.317	6.225	7.689
F3	7.050	8.967	5.842	7.286
SE(m)	0.028	0.031	0.041	
CD (0.05)	0.082	0.090	0.121	

4.1.9 Transpiration rate

The effect of different shade levels and field capacities on the transpiration rate of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 10 . A significant variation in the transpiration rate was observed among the treatments across all the growth stages. The transpiration rate increased up to the flowering stage and then declined at the pod filling stage. A general increase in the transpiration rate was recorded in the plants kept under open conditions, compared to the plants under shade. Among the treatments, the control T7 (open + 100% field capacity) recorded the maximum transpiration rate (5.11 m mol H_2O m^2 s^{-1}, 10.04 m mol H_2O m^2 s^{-1} and 7.75 m mol H_2O m^2 s^{-1}) at vegetative, flowering and pod filling stages respectively. A significant reduction in the transpiration rate was observed in the treatment T3 (50% shade + 50% field capacity) in all the three growth stages studied (3.49 m mol H_2O m^2 s^{-1}, 5.36 m mol H_2O m^2 s^{-1} and 2.96 µ mol H_2O m^2 s^{-1} respectively), whereby the reduction was to an extent of 48.43%, compared to the control, T7. It was noticed that in the flowering stage, T7 and T8 (open + 100% and 75% field capacity) were found to be statistically on-par with each other; whereas T1 (50% shade + 100% field capacity) was on-par with T2 (50% shade + 75% field capacity). Among the shade levels, open condition (S3) accounted for the highest value of transpiration rate in all the growth stages studied. Among moisture stress levels, 100% field capacity (F1) resulted in highest values for the transpiration rate in all the growth stages studied.

Table 10. Effect of different shade levels and moisture levels on transpiration rate (m mol H_2O m^{-2} s^{-1}) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	3.74	7.00	3.73	4.82
T2=S1F2	3.64	6.41	3.49	4.51
T3=S1F3	3.49	5.36	2.96	3.94
T4=S2F1	4.12	8.31	4.56	5.66
T5=S2F2	4.29	8.05	4.34	5.56
T6=S2F3	4.38	7.98	4.18	5.51
T7=S3F1	5.11	10.04	7.75	7.63
T8=S3F2	4.99	9.99	6.84	7.27
T9=S3F3	4.84	9.67	6.70	7.07
SE(m)	0.019	0.023	0.015	
CD (0.05)	0.055	0.068	0.045	
S1	3.619	6.255	3.391	4.422
S2	4.283	8.113	4.357	5.584
S3	4.980	9.899	7.096	7.325
SE(m)	0.011	0.013	0.009	
CD (0.05)	0.032	0.039	0.026	
F1	4.346	8.450	5.345	6.047
F2	4.303	8.147	4.888	5.779
F3	4.234	7.670	4.611	5.505
SE(m)	0.011	0.013	0.009	
CD (0.05)	0.032	0.039	0.026	

4.2. BIOCHEMICAL PARAMETERS

4.2.1 Chlorophyll content

4.2.1.1 Chlorophyll ‘a’ content

The effect of different shade levels and field capacity levels on chlorophyll ‘a’ content of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 11 . A significant variation in the chlorophyll ‘a’ content was observed among the treatments across all the growth stages. A general increase in chlorophyll ‘a’ content was recorded in the plants kept under shade, compared to the plants under open condition. Among the treatments, T6 (25% shade+ 50% field capacity) recorded the maximum chlorophyll ‘a’ content (1.402 mg g^{-1}, 1.501 mg g^{-1} and 1.598 mg g^{-1}) at vegetative, flowering and pod filling stages respectively, up to a degree of 48.11%. A significant reduction in chlorophyll ‘a’content was observed in the control treatment T7 (open + 100% field capacity) in all the three growth stages studied (0.915 mg g^{-1}, 1.013 mg $g^{-1}$and 1.111 mg g^{-1}respectively). It was observed that in the vegetative stage, T5 (25% shade+ 75% field capacity) was statistically on-par with T3 (50% shade+ 50% field capacity). In the flowering stage, T9 (open+ 50% field capacity) was statistically on-par with T8 (open+ 75% field capacity); whereas T8 was on-par with T7. In the pod filling stage, T5 was found to be on-par with T3 and T4 (25% shade+ 100% field capacity); T9 was on-par with T8 and T7; whereas T8 was statistically on-par with T7. Among the shade levels, 25% shade (S2) accounted for the highest value of chlorophyll ‘a’ content in all the growth stages, which favoured the chlorophyll ‘a’ content by 30.25% in comparison to the control level, open (S3). Among moisture stress levels, 50% field capacity (F3) resulted in highest values for chlorophyll ‘a’ content in all the growth stages studied, enhancing the chlorophyll ‘a’ content by 6.72%, compared to the control level, 100% field capacity (F1).

Table 11. Effect of different shade levels and moisture levels on chlorophyll 'a' content (mg g^{-1}) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	1.221	1.323	1.421	1.322
T2=S1F2	1.265	1.367	1.465	1.366
T3=S1F3	1.171	1.273	1.371	1.272
T4=S2F1	1.138	1.240	1.338	1.239
T5=S2F2	1.178	1.280	1.378	1.279
T6=S2F3	1.402	1.501	1.598	1.500
T7=S3F1	0.915	1.013	1.111	1.013
T8=S3F2	0.928	1.030	1.128	1.029
T9=S3F3	0.941	1.043	1.141	1.042
SE(m)	0.010	0.008	0.014	
CD (0.05)	0.030	0.024	0.042	
S1	1.219	1.321	1.419	1.320
S2	1.239	1.340	1.438	1.339
S3	0.928	1.029	1.127	1.028
SE(m)	0.006	0.005	0.014	
CD (0.05)	0.017	0.014	0.042	
F1	1.091	1.192	1.290	1.191
F2	1.124	1.226	1.324	1.225
F3	1.171	1.272	1.370	1.271
SE(m)	0.006	0.005	0.014	
CD (0.05)	0.017	0.014	0.042	

4.2.1.2 Chlorophyll 'b'

The effect of different shade levels and field capacity levels on chlorophyll 'b' content of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 12 . A significant variation in the chlorophyll 'b' content was observed among the treatments across all the growth stages. The chlorophyll 'b' content was found to decrease with decreasing field capacities under open conditions. A general increase in chlorophyll 'b 'content was recorded in the plants kept under shade, compared to the plants under open condition. Among the treatments, T6(25% shade+ 50% field capacity) recorded the maximum chlorophyll 'b' content (0.349 mg g^{-1}, 0.45 mg g^{-1} and 0.547 mg g^{-1}) at vegetative, flowering and pod filling stages respectively, which is a massive increase of 157.36%, compared to the control, T7 (open + 100% field capacity). A significant reduction in chlorophyll 'b' content was observed in the treatment T9 (open + 50% field capacity) in all the three growth stages studied (0.051 mg g^{-1}, 0.107 mg $g^{-1}$and 0.205 mg g^{-1}respectively), to a degree of 30.59% in comparison to the control. It was observed that in the vegetative stage, T1 (50% shade+ 100% field capacity) and T2 (50% shade+ 75% field capacity) were statistically on-par with each other; whereas T7 was on-par with T8 (open + 75% field capacity). However, for the flowering and pod filling stages, T6 (25% shade+ 50% field capacity) was statistically on-par with T3(50% shade+ 50% field capacity); T1 was on-par with T2; T4(25% shade+ 100% field capacity) was on-par with T5(25% shade+ 75% field capacity); whereas T7 and T8 were statistically on-par with each other. Among the shade levels, 50% shade (S1) accounted for the highest value of chlorophyll 'b' content in all the growth stages, enhancing the chlorophyll b content by an enormous 166.09%, compared to the control level, open (S3). Among moisture stress levels, 50% field capacity (F3) resulted in highest values for chlorophyll 'b' content in all the growth stages studied, boosting the chlorophyll b content by 14.37% in comparison to the 100% field capacity (F1).

Table 12. Effect of different shade levels and moisture levels on chlorophyll ‘b’ content (mg g^{-1}) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	0.298	0.400	0.498	0.399
T2=S1F2	0.293	0.395	0.493	0.394
T3=S1F3	0.340	0.442	0.540	0.441
T4=S2F1	0.210	0.312	0.410	0.311
T5=S2F2	0.201	0.303	0.401	0.302
T6=S2F3	0.349	0.450	0.547	0.449
T7=S3F1	0.075	0.175	0.273	0.174
T8=S3F2	0.069	0.169	0.267	0.168
T9=S3F3	0.051	0.107	0.205	0.121
SE(m)	0.003	0.004	0.004	
CD (0.05)	0.007	0.011	0.012	
S1	0.310	0.412	0.510	0.411
S2	0.253	0.355	0.453	0.354
S3	0.065	0.15	0.248	0.154
SE(m)	0.001	0.002	0.002	
CD (0.05)	0.004	0.006	0.007	
F1	0.194	0.296	0.394	0.295
F2	0.188	0.289	0.387	0.288
F3	0.247	0.333	0.431	0.337
SE(m)	0.001	0.002	0.002	
CD (0.05)	0.004	0.006	0.007	

4.2.1.3 Chlorophyll a: b ratio

The effect of different shade levels and field capacity levels on chlorophyll a: b ratio of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 13. A significant variation in the chlorophyll a: b ratio was observed among the treatments across all the growth stages. The chlorophyll a: b ratio was found to gradually decreasing across the growth stages. A significant increase in chlorophyll a: b ratio was observed for the plants kept under open condition compared to the plants under artificial shaded condition. Among the treatments, T9 (open + 50% field capacity) recorded the maximum chlorophyll a: b ratio (18.45, 9.748 and 5.569) for vegetative, flowering and pod filling stages respectively, to an extent of 53.06% in comparison to the control, T7 (open + 100% field capacity). A significant reduction in the chlorophyll a: b ratio was observed in the treatment T3 (50% shade + 50% field capacity) for all the three growth stages (3.444, 2.882 and 2.54 respectively) studied, which diminished the chlorophyll a: b ratio by 59.81%, compared to the control, T7. It was observed that in the vegetative stage, T1 (50% shade + 100% field capacity) was statistically on-par with T6 (25% shade + 50% field capacity); whereas T2 (50% shade + 75% field capacity) was on-par with T1. In the flowering stage, the statistically on-par pairs were, T2 and T6; T6 and T1. In the pod filling stage, T8 and T7; T2, T6 and T1; T6 and T1 were the statistically on-par pairs. Among the shade levels open condition (S3) accounted for the highest value of chlorophyll a: b ratio, in all the growth stages. Among moisture stress levels, 50% field capacity (F3) resulted in the highest values for chlorophyll a: b ratio in all the growth stages studied, causing an increase of 17.63% over the control level, 100% field capacity (F1).

Table 13. Effect of different shade levels and moisture levels on chlorophyll a: b ratio of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	4.099	3.308	2.853	3.420
T2=S1F2	4.321	3.462	2.972	3.585
T3=S1F3	3.444	2.882	2.54	2.955
T4=S2F1	5.419	3.974	3.265	4.219
T5=S2F2	5.865	4.225	3.436	4.509
T6=S2F3	4.018	3.338	2.923	3.426
T7=S3F1	12.201	5.789	4.071	7.354
T8=S3F2	13.449	6.097	4.228	7.925
T9=S3F3	18.45	9.748	5.569	11.256
SE(m)	0.081	0.046	0.058	
CD (0.05)	0.239	0.134	0.169	
S1	3.955	3.217	2.789	3.320
S2	5.101	3.846	3.208	4.052
S3	14.700	7.211	4.623	8.845
SE(m)	0.047	0.026	0.033	
CD (0.05)	0.138	0.078	0.098	
F1	7.240	4.357	3.396	4.998
F2	7.878	4.595	3.545	5.339
F3	8.637	5.323	3.677	5.879
SE(m)	0.047	0.026	0.033	
CD (0.05)	0.138	0.078	0.098	

4.2.1.4 Total chlorophyll

A significant variation in the total chlorophyll content was observed among the treatments across all the growth stages. The effect of different shade levels and field capacity levels on total chlorophyll content of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 14. A general increase in total chlorophyll content was recorded in the plants kept under shade, compared to the plants under open condition. Among the treatments, T6 (25% shade+ 50% field capacity) recorded the maximum total chlorophyll content (1.747 mg g^{-1}, 1.947 mg g^{-1} and 2.141 mg g^{-1}) at vegetative, flowering and pod filling stages respectively, favouring the total chlorophyll content up to 64.18%,compared to that of the control, T7 (open+ 100% field capacity). A significant reduction in total chlorophyll content was observed in the treatment T9 (open + 50% field capacity) in all the three growth stages studied (0.962 mg g^{-1}, 1.151 mg $g^{-1}$and 1.347 mg g^{-1}respectively), the reduction amounting to 2.64% in comparison to the control,T7. It was observed that in the vegetative stage, T2(50% shade+ 75% field capacity) was statistically on-par with T3(50% shade + 50% field capacity); T3 was on-par with T5(25% shade+ 75% field capacity); T5 was on-par with T4(25% shade+ 100% field capacity); whereas T7 was statistically on-par with T9. In the flowering stage, T3 was statistically on-par with T5; T5 with T4, T8 with T7; whereas T7 was statistically on-par with T9. Among the shade levels, 25% shade (S2) accounted for the highest value of total chlorophyll content in vegetative and pod filling stages, by 45.84% and 37.54% respectively; whereas 50% shade (S1) contributed to the highest total chlorophyll content in flowering stage, by 47.67% in comparison to the control level, open (S3). Among moisture stress levels, 50% field capacity (F3) resulted in highest values for total chlorophyll content in all the growth stages studied, to an extent of 5.66% in comparison to the control level, 100% field capacity (F1). It was noticed that the field capacities, F2 (75% field capacity) and F1 were statistically on-par in the vegetative stage; F3 and F2 were on-par in the flowering stage; whereas F1 and F2 were statistically on-par in the pod filling stage.

Table 14. Effect of different shade levels and moisture levels on total chlorophyll content (mg g^{-1}) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	1.515	1.727	1.915	1.719
T2=S1F2	1.427	1.89	1.83	1.716
T3=S1F3	1.403	1.607	1.803	1.604
T4=S2F1	1.346	1.55	1.746	1.547
T5=S2F2	1.375	1.587	1.775	1.579
T6=S2F3	1.747	1.947	2.141	1.945
T7=S3F1	0.992	1.183	1.379	1.185
T8=S3F2	1.110	1.203	1.391	1.235
T9=S3F3	0.962	1.151	1.347	1.153
SE(m)	0.015	0.016	0.020	
CD (0.05)	0.043	0.047	0.06	
S1	1.448	1.741	1.849	1.679
S2	1.489	1.695	1.887	1.690
S3	1.021	1.179	1.372	1.191
SE(m)	0.008	0.009	0.012	
CD (0.05)	0.025	0.027	0.035	
F1	1.284	1.487	1.68	1.484
F2	1.304	1.56	1.665	1.510
F3	1.371	1.568	1.764	1.568
SE(m)	0.008	0.009	0.012	
CD (0.05)	0.025	0.027	0.035	

4.2.2 Total soluble protein

The effect of different shade levels and field capacity levels on total soluble protein content of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub. is presented in Table 15 . A significant variation in the total soluble protein content was observed among the treatments across all the growth stages. The total soluble protein was found to decrease with decreasing field capacities under open conditions. A general increase in total soluble protein content was recorded in the plants kept under open conditions, compared to the plants under shade. Among the treatments, control T7 (open+ 100% field capacity) recorded the maximum total soluble protein content (1.18 mg g^{-1}, 2.92 mg g^{-1} and 4.64 mg g^{-1}) at vegetative, flowering and pod filling stages respectively. A significant reduction in total soluble protein content was observed in the treatment T3 (50% shade + 50% field capacity) in all the three growth stages studied (0.62 mg g^{-1}, 1.65 mg $g^{-1}$and 2.65 mg g^{-1}respectively), up to an extent of 43.71%, compared to the control (T7). It was noticed that in the vegetative stage, T5 (25% shade + 75% field capacity) and T7 were statistically on-par; T6 (25% shade + 50% field capacity) and T8 (open + 75% field capacity) were on-par; whereas T2 (50% shade + 75% field capacity) and T4 (25% shade + 100% field capacity) were on-par with each other. In the flowering stage, T8 and T5 were found to be statistically on-par treatments. In the pod filling stage, T8 was on-par with T5; whereas T6 and T9 (open + 50% field capacity) were statistically on-par with each other. Among the shade levels, open condition (S3) accounted for the highest value of total soluble protein content across all the growth stages. Among moisture stress levels, 75% field capacity (F2) resulted in highest values for total soluble protein content in vegetative and flowering stages, favouring the total soluble protein content by 3.93% in comparison to the control level, F1 (100% field capacity). On the other hand, 100% field capacity (F1) resulted in the highest value for total soluble protein in pod filling stage.

Table 15. Effect of different shade levels and moisture levels on total soluble protein content (mg g^{-1}) of *Cyamopsis tetragonoloba* (L.) Taub.

Treatment	Vegetative stage	Flowering Stage	Pod filling stage	Mean
T1=S1F1	0.83	2.23	3.53	2.20
T2=S1F2	0.73	2.04	3.32	2.03
T3=S1F3	0.62	1.65	2.65	1.64
T4=S2F1	0.71	1.85	3.05	1.87
T5=S2F2	1.18	2.51	3.86	2.52
T6=S2F3	1.12	2.48	3.82	2.47
T7=S3F1	1.18	2.92	4.64	2.91
T8=S3F2	1.11	2.52	3.87	2.50
T9=S3F3	1.06	2.44	3.8	2.43
SE(m)	0.008	0.009	0.007	
CD (0.05)	0.025	0.027	0.021	
S1	0.724	1.973	3.167	1.955
S2	1.001	2.281	3.575	2.286
S3	1.116	2.625	4.103	2.615
SE(m)	0.005	0.005	0.004	
CD (0.05)	0.014	0.015	0.012	
F1	0.903	2.332	3.738	2.324
F2	1.007	2.355	3.684	2.349
F3	0.932	2.192	3.422	2.182
SE(m)	0.005	0.005	0.004	
CD (0.05)	0.014	0.015	0.012	

Discussion

5. DISCUSSION

The current investigation on the "Physiological and biochemical studies in cluster bean [*Cyamopsistetragonoloba*(L.) Taub.] as influenced by light and moisture stress" was carried out at the Department of Plant Physiology, College of Agriculture, Vellayani, during 2018-2020. The findings of the study are discussed in this chapter. A critical discussion on the outcome of the study with the support of relevant references based on experimental evidence has been done in the following pages of this chapter.

Cluster bean is an important commercial crop, highly valued for its best yield, greater nutritional and industrial importance and drought tolerance. In India, it is extensively cultivated during kharif season. Cluster bean is a versatile and multi-purpose legume crop, cultivated mostly as vegetable, forage, and cover crop (Arora and Pahuja, 2008). The tender pods and seeds of the crop have greater economic value.

It is a drought hardy leguminous crop because of its deep tap root system and has high capacity to recover from water stress. Cluster bean is the most important drought resistant kharif legume capable of growing under poor fertility and scanty rainfall and grown mainly in arid and semi-arid tracts of India. The importance of the yield loss, both in terms of quality and quantity would usually depend on the response of cluster bean varieties to drought stress. Improving water access and management are practically challenging, since water is a scarce resource and thus drought response is a crucial aspect to be analysed.

Growth and development of crop plants are affected by the quantity, quality and the duration of sunlight, as the light energy is the main input of the photosynthetic process in the green plants (Noggle and Fritz, 1979; Zelitch, 1971). Despite the fact that different crop species have differential growth and yield response to light intensity in the course of their ontogeny, growth and yield stability of a crop under low light condition has greater significance from the view point of physiological adaptability (Zelitch, 1971). Studies related to the effect of various abiotic stresses on the growth and metabolism of cluster bean may be useful to evaluate the genotypes most suitable for such situations.

Hence the current study was carried out to investigate the influence of abiotic stress factors like light and moisture stress on the physiological and biochemical changes taking place in *Cyamopsis tetragonoloba*(L.) Taub.

5.1 EFFECT OF LIGHT AND MOISTURE STRESS ON PHYSIOLOGICAL PARAMETERS

Abiotic stress *viz*. moisture stress and light stress were imposed on *C.tetragonoloba*(L.) Taub. for a period spanning from two weeks after sowing to the pod filling stage. The treatments were given in combinations of three levels of moisture at 100%, 75% and 50% field capacity and three levels of light intensities at 50%, 25% shade and open condition. Observations on the physiological parameters like plant height, number of primary branches per plant, root dry weight, shoot dry weight, root: shoot ratio, crop growth rate, relative growth rate, photosynthetic and transpiration rate were taken. The influence of moisture stress and light stress and their interaction on growth parameters is discussed. Shading induced alleviation of the adverse effects of drought may depend on the shading intensity (*viz.* moderate shading can mitigate the negative effects of drought, while severe shading can aggravate these effects).

In the present study, drought or shading-induced reduction in growth was aggravated under the combined influence of shading and drought which reduced plant height up to 13.61% and 12.1% for treatments T2 (50% shade + 75% field capacity) and T3 (50% shade + 50% field capacity) respectively in comparison to the control T7(open+ 100% field capacity). The treatments T2 and T3 were statistically on-par with each other, as far as the reduction in plant height is concerned.

Moisture stress had a detrimental effect on plant height in comparison to shading. The results verified that with decreasing field capacities, the decline in plant height was more visible, with the reduction being most significant for F3 (50% field capacity) which reduced the plant height up to 7.6% compared to F1 (100% field capacity). However, moderate shade level of S2 (25% shade) showed an increase in plant height by 14.1% compared to S3 (open condition).But severe shading of S1 (50% shade) down-regulated the plant height by 2.88%. Hence moderate shade level of 25% was found to favour the plant height.

Thus the plant height was found to be distinctively influenced by low light. However an increasing trend in plant height was observed under shaded condition as compared to open condition. This outcome is in accord with the results in *Amaranthus viridis* reported by Farrukh *et al*. (2003). In the present study, the plant height decreased with decreasing irrigation frequencies. These results were similar to the findings by Chauhan and Abugho (2013), who reported a similar decreasing trend with decreasing field capacities in *Amaranthus spinosus*, *Leptochloa chinensis*, and rice.

Jensen *et al.* (1998) opined that auxin transport plays an important role in stem elongation and the rate of transport is reliant on light intensity. Shading result in a change of gibberellin concentration in plants, which in turn result in increased plant height under shade condition. Increase in plant height is the outcome of phytochrome faciliated shade avoidance mechanism of the plants as reported by Lambers *et al*. (1998). The primary plant process altered by moisture stress is the cell elongation which is the main cause for lowered plant height under moisture stress (Taiz and Zeiger, 2010).

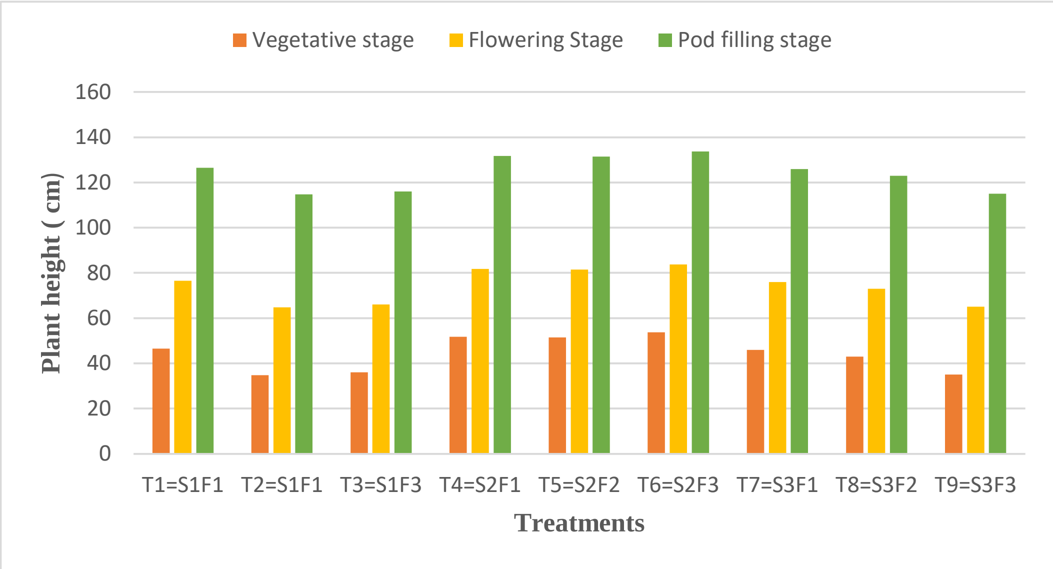

Fig.1. Effect of different shade levels and moisture levels on plant height of *Cyamopsis tetragonoloba*(L.) Taub.

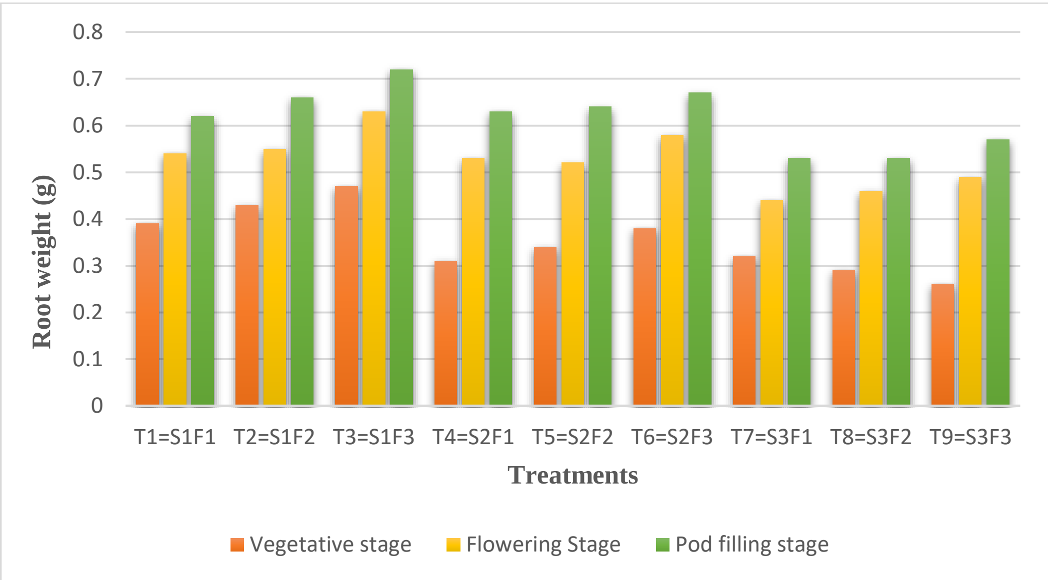

Fig.2. Effect of different shade levels and moisture levels on root weight of *C. tetragonoloba* (L.) Taub.

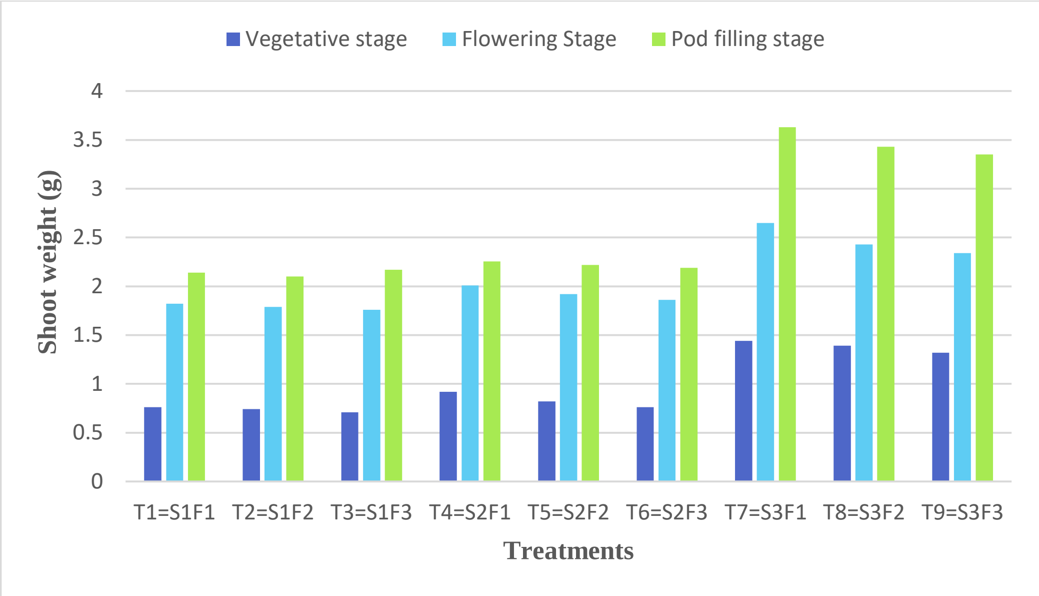

Fig.3. Effect of different shade levels and moisture levels on shoot weight of *Cyamopsis tetragonoloba* (L.) Taub.

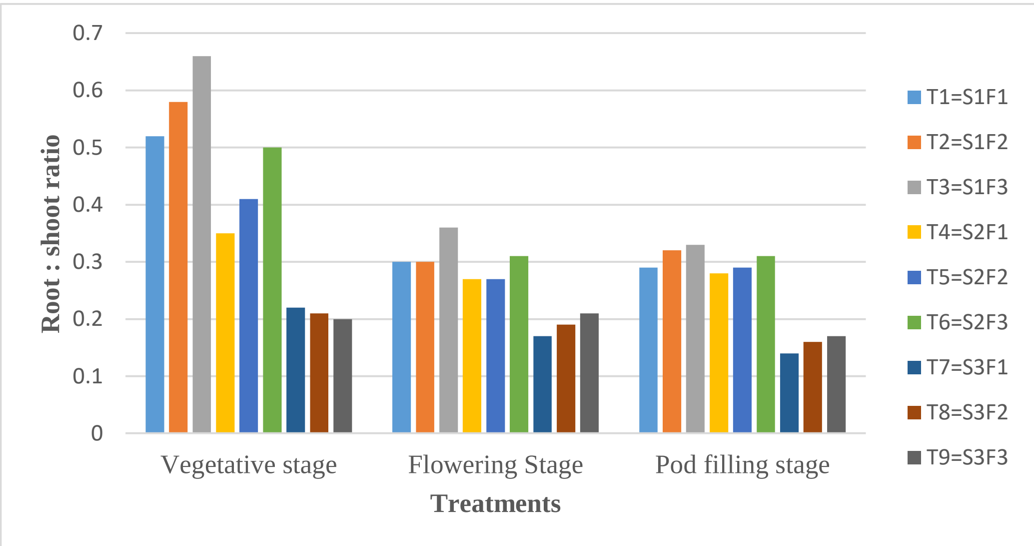

Fig.4. Effect of different shade levels and moisture levels on root: shoot ratio of *C. tetragonoloba* (L.) Taub.

The number of primary branches per plant was significantly affected only in the case of flowering stage. The combined light and moisture stress resulted in the reduction of the number of primary branches per plant for all treatments across all the growth stages studied. In the flowering stage, the reduction was most evident for treatment T3 (50% shade + 50% field capacity), followed by T5 (25% shade + 75% field capacity) where a reduction of 47.06% and 41.18% was observed compared to that of control, T7 (open + 100% field capacity).Light stress levels of S1 (50% shade) and S2 (25% shade) showed reduction in the number of primary branches per plant by 32.46% and 25% respectively, compared to the control, S3 (open). Moisture stress levels also had a detrimental effect in vegetative and flowering stages. In vegetative stage, F2 (75% field capacity) and F3 (50% field capacity) showed a reduction of 11.88% and 18.65%; whereas in flowering stage, F2 and F3 resulted in a decline of 13.91% and 18.99% respectively, in comparison to the control, F1 (100% field capacity).

The number of primary branches per plant was reduced under shaded conditions. It was found that the number of primary branches were less under shade as compared to open condition. The finding was in agreement with the results in *Rosa damascena* Mill. reported by Thakur *et al*., (2019). In the present study the number of primary branches per plant decreased with decreasing field capacities. These findings were consistent with the findings of Alamin (2018), who reported a similar decreasing trend with decreasing irrigation frequencies in *Brassica napus* L.

According to Kumar *et al.*, (2014) the number of primary branches might be higher under open conditions due to higher level of Photosynthetic photon flux density. A low light intensity usually inhibits plant growth and productivity by affecting the gas exchange. Giri (2001) also reported that in Mustard, regular irrigations gave the highest number branches per plant and the lowest number of branches per plant was found in case of without irrigation. Probably irrigation water supported the plant to initiate more branches and it was severely affected with decreasing field capacities.

The root dry weight showed an increase under the combined light and moisture stress for almost all treatments across all the growth stages in comparison to control, T7 (open + 100% field capacity). However the increase was statistically significant only for vegetative and flowering stages. In vegetative stage, the root dry weight was up regulated for T3 (50% shade + 50% field capacity) and T2 (50% shade + 75% field capacity) to an extent of 46.88% and 34.38% respectively, whereas there was a slight decrease of 3.13% in root dry weight for T4 (25% shade + 100% field capacity) in comparison to control, T7. In the flowering stage, the root dry weight was up regulated for T6 (25% shade + 50% field capacity) and T3 (50% shade + 50% field capacity) by 43.18% and 31.82% in comparison to control, T7.Light stress and moisture stress individually had a positive impact on the root dry weight across all the growth stages. Light stress levels of S1 (50% shade) and S2 (25% shade) improved the root dry weight by 29.16% and 18.41% respectively in comparison to control. Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) increased the root dry weight by 2.37% and 10.8% respectively, compared to control, F1 (100% field capacity).

Root weight showed significant variation among the treatments except for pod filling stage. It was found that the root weight was higher under shade as compared to open condition. However, Carneiro *et al* ., (2015) reported that when shaded *Jatropha curcas* plants were exposed to long-lasting cycles of moisture stress, biomass allocation to roots was lowered by more than 40% but there was no change in root length under water stress, so access to the soil water was upheld. In the present study, combined light and water stress resulted in an increase in root weight up to 40% (T3: 50% shade +50% field capacity) for 50% shade and up to 26% (T6: 25% shade + 50% field capacity) for 25% shade level. In the present study the root weight increased with decreasing field capacity. This result was in congruent with the findings of Kumar (2005), who reported a similar increase in root weight under soil moisture stress in mothbean.

The root weight might have increased under combined effect of shade and moisture stress along the same line of increase which was observed with that of moisture stress. According to Kumar (2005), the increase in root weight under soil moisture stress might be due to initiation of more roots and increased root length to extract more water from deeper soil profiles.

The effect of the combined light and moisture stress on the shoot dry weight was significant for the treatments across the growth stages under study. It was found that the stresses in combination led to the decrease in shoot dry weight for the different treatments, in comparison to the control, T7 (open + 100% field capacity). It was found that the reduction was the most evident in treatment T3 (50% shade + 50% field capacity) and T2 (50% shade + 75% field capacity), to an extent of 40.03% and these two treatments were statistically on-par, across all the growth stages. Light and moisture stress separately had a significant effect on the shoot dry weight across the different critical growth stages studied. Light stress levels of S1 (50% shade) and S2 (25% shade) reduced the shoot dry weight by 36.34% and 31.97% respectively, compared to control, S3 (open). Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) decreased shoot dry weight by 4.45% and 6.49% respectively, in comparison to control, F1 (100% field capacity).

A significant variation in shoot weight was observed among the treatments for all the growth stages studied. It was found that the shoot weight was less under shade as compared to open condition. This finding is in line with that of Wu *et al.*, (2017) who found significant reduction in shoot biomass in shade treated plants compared to those plants grown under full sunlight, in Soybean. In the present study the shoot weight decreased with decreasing field capacities. These results were in congruent with the findings of Kumar (2005), who reported a similar decrease in shoot weight under soil moisture stress in mothbean.

The distinct reduction in the leaf dry matter in shade grown plants might be due to the reason that leaf thickness is regulated by light and that high irradiance produces the thickest leaves. Thicker leaves have more photosynthetically active cells, whereby the enlargement of mesophyll thickness make the photosynthetic rate to raise and subsequently results in higher biomass production in sun grown plants (Fails et al 1982; Fetcher et al 1983). Therefore, thinner leaves don’t have a strong capacity of photosynthetic and biomass accumulation and hence the possible reduction in shoot biomass, under shade. The shoot and total dry weight reduction under moisture stress situation in this study might be due to the reduction in values of growth relating parameters like leaf and stem. Several workers also found lower values of many growth parameters including dry weight of different plant parts as a result of water stress. (Ayob, 1986; Beese and Moshrefi, 1985; Hedge, 1989; Smittle *et al*., 1994).

A significant variation in root: shoot ratio was observed among the treatments for only the vegetative stage. In the vegetative stage, the combined light and moisture stress resulted in an increase in the root: shoot ratio in T3 (50% shade + 50% field capacity) and T2 (50% shade + 75% field capacity) by an enormous 200% and 163.64% respectively, compared to the control, T7 (open + 100% field capacity). Light stress had a positive impact on the root : shoot ratio, with shade levels, S1(50% shade) and S2 (25% shade) improving the root : shoot ratios by 120.65% and 80.07% respectively, over the control level, S3 (open). Moisture stress levels in terms of F2 (75% field capacity) and F3 (50% field capacity) enhanced the root: shoot ratio by 7.97% and 20.69% respectively, over the control level, F1 (100% field capacity). The root: shoot ratio was higher under shaded conditions compared to that under open conditions. This finding was in agreement with that of Fu *et al.*, (2020) who reported higher root: shoot ratios in the shade compared to non-shade conditions in *Lolium perenne.* In the present study the root: shoot ratio increased with decreasing field capacities. These results were in congruent with the findings of Saidi *et al*., (2010) who observed that in wheat seedlings, the ratio of root weight to shoot weight is augmented with a reduction in soil moisture and also that root growth itself can be boosted under conditions of comparitively reduced soil moisture.

In the present study, the higher root: shoot ratios under shaded conditions might be due to the comparative increase of root weight and the subsequent decline in shoot weight under shaded conditions. The impact of shade on root weight was less severe compared to that of shoot weight. As far as the increase in root weight under decreasing field capacities is concerned, growth response of roots to the reduction in soil moisture was less marked than that of shoots. Hence, the ratio of root weight to shoot weight increased with reduction in the soil water potential (Saidi *et al.,* 2010).

The effect of the combined light and moisture stress on the crop growth rate was significant for the treatments across all the growth stages under study. It was found that the stresses in combination led to the decrease in CGR for the different treatments, in comparison to the control, T7 (open + 100% field capacity). It was observed that the reduction was the most apparent in treatment T3 (50% shade + 50% field capacity) and T2 (50% shade + 75% field capacity), to a degree of 52.68% and 50% respectively across all the growth stages. Light and moisture stress distinctly had a significant effect on the CGR across the different critical growth stages studied. Light stress levels of S1 (50% shade) and S2 (25% shade) diminished the CGR by 47.87% and 39.73% respectively, compared to control, S3 (open). Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) decreased CGR by 6.05% and 8.19% respectively, in comparison to control, F1 (100% field capacity).

A significant variation in the crop growth rate was observed among the treatments for all the growth stages. The crop growth rate increased up to the flowering stage and then declined at the pod filling stage (except under open conditions). The crop growth rate was lower under shaded conditions compared to that of open conditions, which was in line with the results obtained by Lakshmanakumar and Guru (2014) who reported maximum and significantly higher crop growth rate under full sunlight, which reduced significantly with the progressive increase in shade levels across all the crop growth stages in wheat. The same decreasing trend was observed for crop growth rate with decreasing field capacities. Similar results were obtained by Pandey *et al.*, (2000) who found that deficit irrigation modestly reduced the CGR during early vegetative stage, while the reduction was severe under late vegetative and reproductive growth stages in maize.

The CGR is an index exhibiting the increase in dry mass per unit leaf area and per unit land, thus it shows the real growth rate of crop canopy under any condition. In this study, the CGR of plants decreased by shading, in which the solar radiation decreased up to 50% of natural light. This is similar to the finding of

Chaturvedi *et al.*, (1989) in which CGR decreases under shading treatment. Under shaded conditions, leaves are not able to receive enough light for the production of photosynthetic assimilates and they have a reduced ability to produce dry matter. In several studies, Crop Growth Rate (CGR) reduction has been reported as the result of water stress (Hirasawa *et al.*, 1998; Karimi and Sidique, 1991). Water deficit stress through the reduction in the LAI and plants photosynthetic capacity reduces CGR and ultimately total dry matter (Karimi and Sidique, 1991).

The effect of the combined light and moisture stress on the relative growth rate was significant for the treatments across all the growth stages under study. It was found that the stresses in combination led to the drop in RGR for the different treatments, in comparison to the control, T7 (open + 100% field capacity). It was observed that the decline was the most obvious in treatment T3 (50% shade + 50% field capacity) and T2 (50% shade + 75% field capacity), to a degree of 38.59% and 34.7% respectively across all the growth stages. Light and moisture stress alone had a significant effect on the RGR across the different critical growth stages studied. Light stress levels of S1 (50% shade) and S2 (25% shade) lessened the RGR by 31.86% and 23.25% respectively, compared to control, S3 (open). Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) reduced the RGR by 5.06% and 7.64% respectively, in comparison to control, F1 (100% field capacity).

A significant variation in the relative growth rate was observed among the treatments for all the growth stages. The relative growth rate decreased progressively from the vegetative to the pod filling stage. The relative growth rate was lower under shaded conditions compared to that of open conditions, which was in line with the results obtained by Lakshmanakumar and Guru (2014) who found that the maximum and significantly higher RGR was obtained under full sunlight (i.e. control) at all the crop growth stages, which reduced significantly with each successive increase in shades, with no significant difference between 2/3 and 1/3 of full sunlight availability. As far as moisture stress is concerned, the same decreasing trend was observed for relative growth rate with decreasing field capacities. These findings are in congruence with that of Solomon and Labuschagne, (2009) who reported that under moisture stress conditions, RGR declined in the growth stage (45-60 DAP) to the growth period (75-90 DAP) in durum wheat genotypes.

The reduction in RGR under shade might be due to the remobilization of reserves from roots which contribute to above-ground growth early in the growing season along with the allocation of more assimilates to the underground parts(Muthuchelian *et al.,* 1989). The reduction in RGR with decreasing field capacities might be due to slower rate of development and inadequate moisture supply under the stress, with the reduction being significant at the pod filling stage.

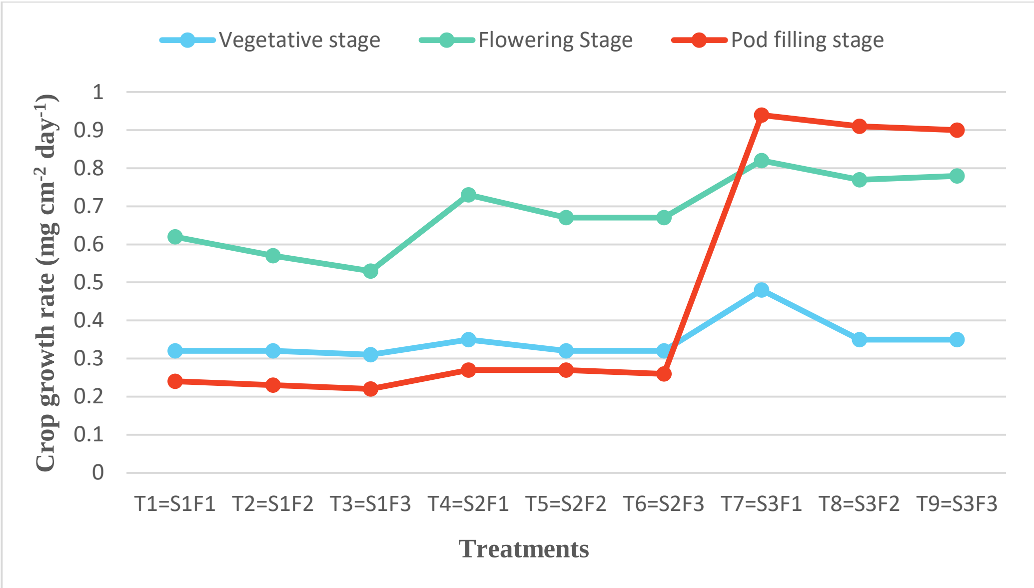

Fig.5. Effect of different shade levels and moisture levels on the crop growth rate of *Cyamopsis tetragonoloba* (L.) Taub.

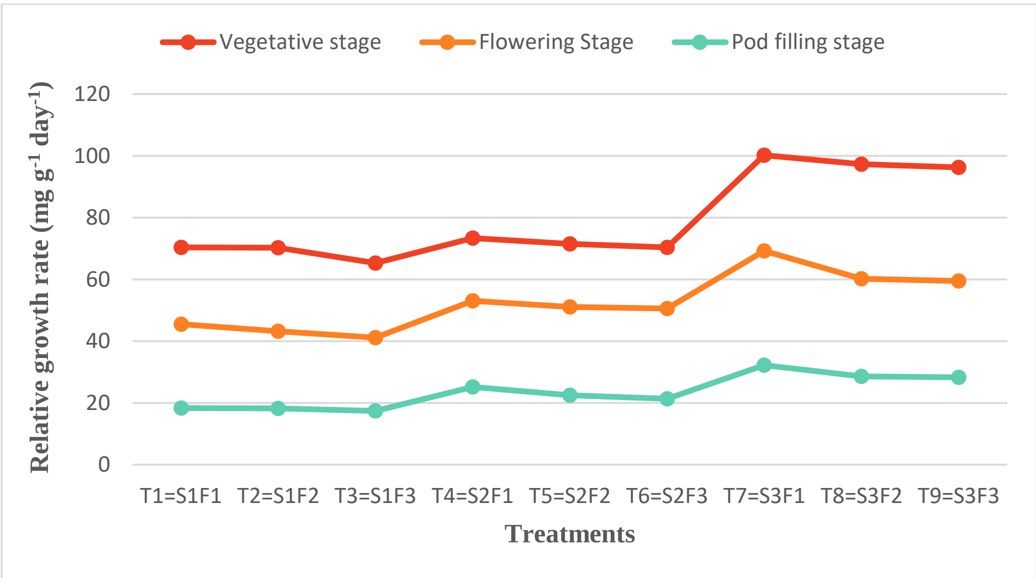

Fig.6. Effect of different shade levels and moisture levels on the relative growth rate of *Cyamopsis tetragonoloba* (L.) Taub.

The impact of the combined light and moisture stress on the photosynthetic rate was significant for the treatments across all the growth stages under study. It was noticed that the stresses in combination led to the decline in photosynthetic rate for the different treatments, in comparison to the control, T7 (open + 100% field capacity). It was noticed that the decline was the most evident in treatment T3 (50% shade + 50% field capacity) and T2 (50% shade + 75% field capacity), to a degree of 58.58% and 53.2% respectively across all the growth stages. Light and moisture stress distinctly had a significant effect on the photosynthetic rate across the different critical growth stages studied. Light stress levels of S1 (50% shade) and S2 (25% shade) lowered the photosynthetic rate by 52.13% and 35.7% respectively, compared to control, S3 (open). Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) reduced the photosynthetic rate by 6.49% and 11.38% respectively, in comparison to control, F1 (100% field capacity).

It was observed that the photosynthetic rate varied significantly across all the growth stages. The photosynthetic rate increased up to the flowering stage and then dropped at pod filling stage. In the present study, higher photosynthetic rate was found in plants growing under open conditions, which is similar to the findings of Myers *et al*., (2005), who reported that leaves of the 0% shade treatment in *Alliaria petiolata* plants had a significantly greater photosynthetic rate than those of the 60% shade treatment plants when measured at 800 µ mol m^{-2} s^{-1} Photosynthetic Photon Flux Density (PPFD).A decreasing trend was also observed for photosynthetic rate with decreasing levels of field capacities, which is in accord with that of Mafakheri *et al*., (2010) who found that the photosynthetic rate decreased in three chickpea cultivars subjected to drought (withholding irrigation) compared to well irrigated(control) plants.

Photosynthesis is the primary physiological process that offers energy and carbon assimilation for plant growth (Lawlor, 2009) but Mittler (2006) stated that it is often repressed and damaged due to its sensitivity to low light stress. The reduction in the net photosynthetic rate in shaded treatments might be due to the reduced stomatal conductance as well as low inter cellular CO_2 concentration. The reason for the decrease in photosynthetic rate in water-stressed plants might be due to the closure of stomata, and reduction in the photosynthetic capacity of mesophyll cells (Zhang *et al*., 2016).

The impact of the combined light and moisture stress on the transpiration rate was significant for the treatments across all the growth stages under study. It was noticed that the stresses in combination led to the decline in transpiration rate for the different treatments, in comparison to the control, T7 (open + 100% field capacity). It was noticed that the decline was the most evident in treatment T3 (50% shade + 50% field capacity) and T2 (50% shade + 75% field capacity), to a degree of 48.43% and 40.87% respectively across all the growth stages, compared to the control. Light and moisture stress distinctly had a major effect on the transpiration rate across the different critical growth stages studied. Light stress levels of S1 (50% shade) and S2 (25% shade) lessened the transpiration rate by 39.64% and 23.76% respectively, compared to control, S3 (open). Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) reduced the transpiration rate by 4.43% and 8.86% respectively, in comparison to control, F1 (100% field capacity).

The effect of the combined shade and field capacity levels on the transpiration rate was found to be significant. Transpiration rate was found to increase up to flowering stage and then it decreased at the pod filling stage. The transpiration rate was found to be higher under open conditions and it decreased with the increasing levels of shade. These results are in agreement with that of Sreekala (1999), who reported a similar decreasing trend in the transpiration rate with increasing shade levels, with the lowest transpiration rate under 80% shade level. Moisture stress resulted in higher values of transpiration rate for plants subjected to 100% field capacity and showed a decline with decreasing field capacities which is similar to the findings of Sreenaiah *et al*., (2015),who reported a decrease in transpiration rates with the increasing drought stress(80%,60% and 30% water) in cluster bean genotypes.

The transpiration rate was found to be inversely proportional to shade level with maximum in open conditions and minimum in 90% shade. Transpiration rate is dependent upon factors like temperature, light, relative humidity and transmittance. Accordingly the rates might be lower under shade nets compared to that of open conditions. (Gaurav *et al*., 2015)

The reduction in transpiration rate under moisture stress might have been due reduced plant water status as well as low stomatal conductance. The lower values of transpiration rate might be attributed to the stomatal aperture which is associated with the regulation of the transpiration rate as reported by Rao and Bhatt (1988).

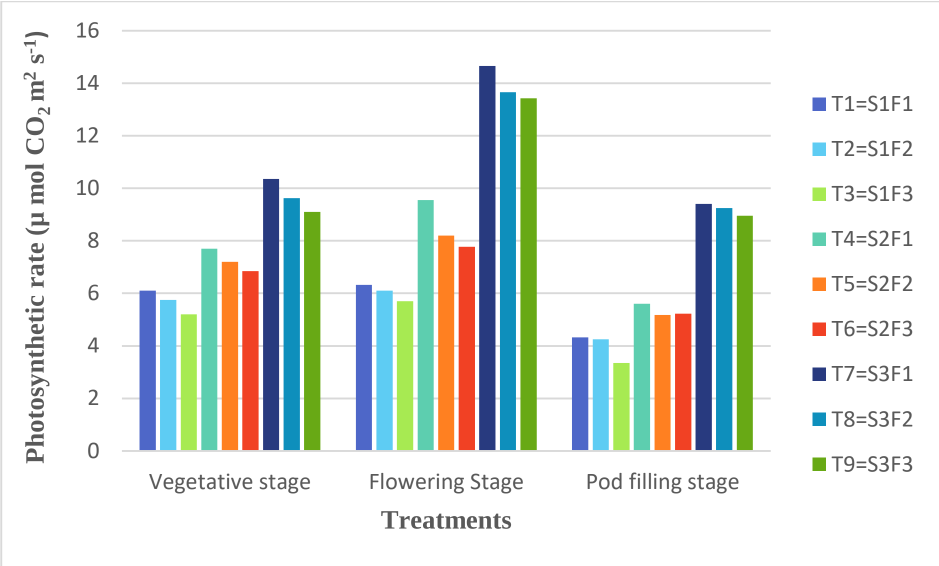

Fig. 7. Effect of different shade levels and moisture levels on the photosynthetic rate of *Cyamopsis tetragonoloba* (L.) Taub.

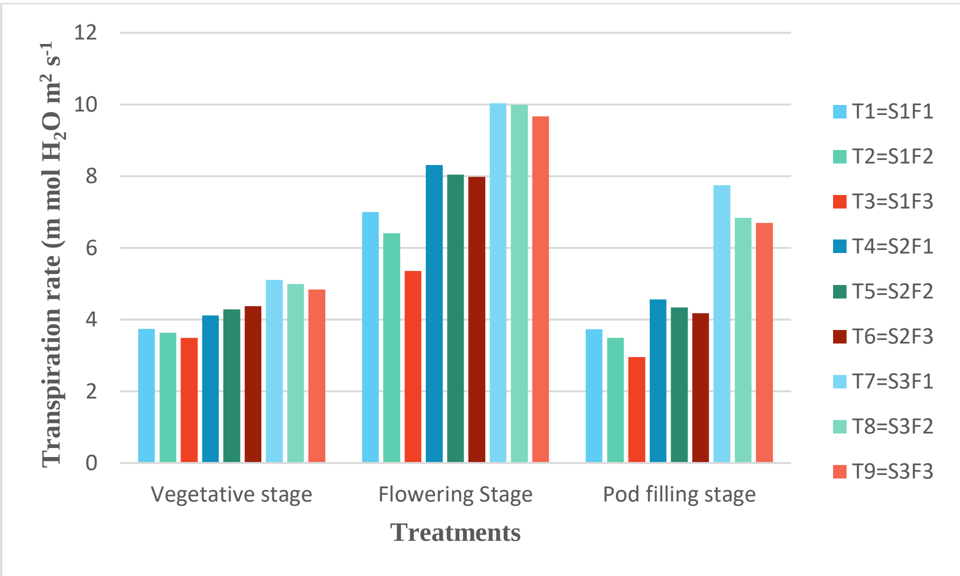

Fig.8. Effect of different shade levels and moisture levels on the transpiration rate of *Cyamopsis tetragonoloba* (L.) Taub.

5.1 EFFECT OF DIFFERENT SHADE LEVELS AND MOISTURE LEVELS ON BIOCHEMICAL PARAMETERS

The effect of light and moisture stress on the biochemical characters such as chlorophyll content and total soluble protein was studied upon. The individual effects of these stresses as well as their interaction effect was considered. Significant differences were observed across all the treatments for the different characters.

5.1.1 EFFECT OF DIFFERENT SHADE LEVELS AND MOISTURE LEVELS ON CHLOROPHYLL CONTENT

The effect of light and moisture stress on the chlorophyll content was studied in terms of chlorophyll a content, chlorophyll b content, chlorophyll a: b ratio and the total chlorophyll content. The present study focussed on the individual effects of light and moisture stress as well as their interaction effect.

The effect of the different treatments on the chlorophyll a content was found to be significant with a general increase in the chlorophyll a in all treatments across all the growth stages studied. Under the influence of both light and moisture stress, chlorophyll a content was enhanced by about 48.11% in T6 (25% shade+ 50% field capacity) and by 34.81% in T2 (50% shade + 75% field capacity). As far as the individual effects are concerned, light stress *i.e.* S1 (50% shade) and S2 (25% shade) resulted in increments of 28.37% and 30.25% in the chlorophyll a content, compared to that of control, S3 (open). Moisture stress in terms of F2 (75% field capacity) and F3 (50% field capacity) however contributed to only 2.64% and 6.20% increase in the chlorophyll a content in comparison to control, F1 (100% field capacity).

Chlorophyll b content was significantly affected by the different treatments across all the growth stages studied. The combination of light and moisture stress, improved chlorophyll b content by an enormous 157.36% in T6 (25% shade+ 50% field capacity) and by 152.77% in T3 (50% shade + 50% field capacity), compared to the control, T7 (open + 100% field capacity). However, under open conditions, chlorophyll b content declined in T8 (open + 75% field capacity) and T9 (open +

50% field capacity) by 3.44% and 30.59% respectively. As far as the individual effects are concerned, light stress *i.e.* S1 (50% shade) and S2 (25% shade) resulted in massive increments of 166.09% and 129.16% in the chlorophyll b content, compared to that of control, S3 (open). Moisture stress in terms of F2 (75% field capacity) and F3 (50% field capacity) however led to a slight decrease of 2.26% and 14.37% increase in the chlorophyll b content in comparison to control, F1 (100% field capacity).

Chlorophyll a: b ratio also varied significantly under the different treatments, with a progressive decrease up to the pod filling stage. Under the influence of both light and moisture stress, chlorophyll a: b ratio was found to decrease significantly in comparison to the control, T7 (open + 100% field capacity). Severe decline in the chlorophyll a: b ratio was observed in T3 (50% shade + 50% field capacity), T1 (50% shade + 100% field capacity) and T6 (25% shade+ 50% field capacity) by about 59.81%, 53.49% and 53.41% respectively. However under open conditions, the chlorophyll a: b ratio was considerably increased by up to 53.06% and a slight increase of 7.76% in T9 (open + 50% field capacity) and T8 (open + 75% field capacity) respectively. The individual effects of the light and moisture stress on the chlorophyll a: b ratio were drastically different to each other. Light stress proved to be detrimental for the chlorophyll a: b ratio with S1 (50% shade) and S2 (25% shade) down regulating the ratio by 62.46% and 54.19% respectively in comparison to the control, S3 (open). However, moisture stress had a positive impact on the chlorophyll a: b ratio with F2 (75% field capacity) and F3 (50% field capacity) enhancing the chlorophyll a: b ratio by 6.84% and 17.63% respectively in comparison to control, F1 (100% field capacity).

The study about the impact of light on growth in *Ageratum conyzoides* ascertained the fact that under shaded conditions there is an increase in Chlorophyll a, Chlorophyll b and total chlorophyll (Sun *et al.*, 2012).The ratio of Chl *a* /Chl *b*, among other indices, has been regarded as a key factor for classifying plants in relation to their shade tolerance (Dai *et al*. 2009); individuals of tolerant shade adapted plant species exhibit a low value for this ratio in comparison to the individuals subjected to high light. This is due to a higher concentration of chlorophyll *b* in shade-adapted plants of shade-tolerant species. Increases in Chl *b* content are most likely due to changes in the organization of both light harvesting and electron transport components (Yamazaki *et al*. 2005; Dai *et al*. 2009). The basis for Chl a/Chl b ratio decrease might be the development of bigger Light Harvesting Complexes by plants under low light availability. Both Chl a and Chl b are found in LHCs but only Chl a is found in the reaction centers. Consequently, bigger LHCs as a response to low light lead to increments of both Chl a and Chl b, nevertheless the overall Chl a/Chl b ratio drops as there is no increase in the number of Chl a molecules forming the reaction centers of the photosystems.

Moisture stressed plants showed a significant reduction in chlorophyll a and b than watered plants in Lettuce (Agami, 2013).However, opposed to this, the results obtained in this study indicate that moisture stress (75% field capacity and 50% field capacity) improves the chlorophyll a content slightly, whereas chlorophyll b content is improved under 50% field capacity level only, and that too a meagre increment.

Increment in the total chlorophyll content can be seen as one of the plant's efforts to adjust to shaded conditions. This phenomenon might be seen as an adaptive behavior to capture the maximum possible light under shade by increasing chlorophyll content through the enhancement of light harvesting complexes. The results of this study reveal that the combination of light and moisture stress seems to boost the total chlorophyll content and the low light conditions in particular accelerates this process.The total chlorophyll content was enhanced under all treatments except that of T9, with increase of up to 64.18% total chlorophyll content in treatment T6 (25% shade+ 50% field capacity) whereas treatment T1 (50% shade+ 100% field capacity) improved the chlorophyll content by 45.1%. Hence it is noteworthy that the combined influence of light and moisture stress is favourable for the enhancement of the total chlorophyll content in Cluster bean. However, in the current study, the detrimental effect of moisture stress on the chlorophyll content could be observed through about 2.64% reduction in mean total chlorophyll content, for treatment T9 (open+50% field capacity). The reduction of chlorophyll content under moisture stress might be due to the fact that water deficit diminishes the chlorophyll content by causing internal modification in the thylakoid membrane. The total chlorophyll content was also increased under the individual stresses but the increment was more evident under light stress than that of moisture stress. Light stress *i.e.* S1 (50% shade) and S2 (25% shade) led to an increase of 41.04% and 41.97% respectively in the total chlorophyll content; whereas moisture stress in terms of F2 and F3 led to a meagre increment of 1.75% and 5.66% in the total chlorophyll content.

There was a general increase in the total chlorophyll content in almost all treatments under the combined effect of light and moisture stress. It was also observed that light and moisture stress separately also improved the total chlorophyll content. The findings of several workers such as Muhidin *et al*., (2018) confirms that shading has a significant effect on the total chlorophyll content and moderate levels of shade (25%) is beneficial for the upland red rice cultivars. Similarly, there have been reports indicating slight increase in total chlorophyll content under drought such as that of Nikolaeva *et al*., (2010) who found an insignificant increase in the total chlorophyll content in wheat cultivars during the first two periods (cessation of watering for 3 and 5 days) of drought, which was later decreased by 13–15%(cessation of watering for 7 days). Reduced or unchanged chlorophyll level during drought stress has been described in many species, based on the duration and severity of drought. Surendar *et al*., (2013) stated that tolerant and moderately tolerant cultivars displayed lesser reduction in total chlorophyll content with 5 and 10 per cent in response to irrigated level of 50% available soil moisture over control.

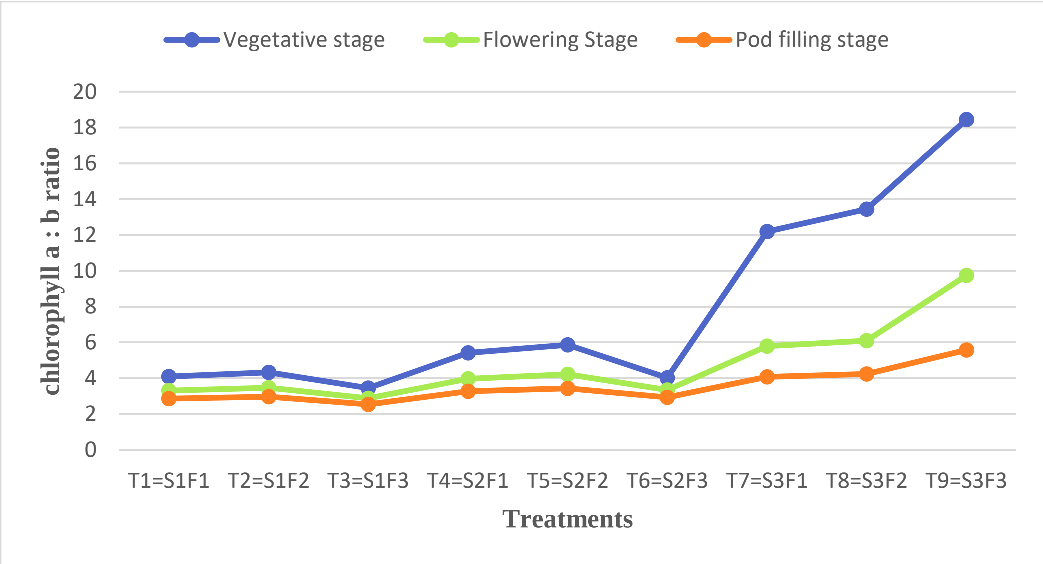

Fig. 9. Effect of different shade levels and moisture levels on chlorophyll a: b ratio of *Cyamopsis tetragonoloba* (L.) Taub

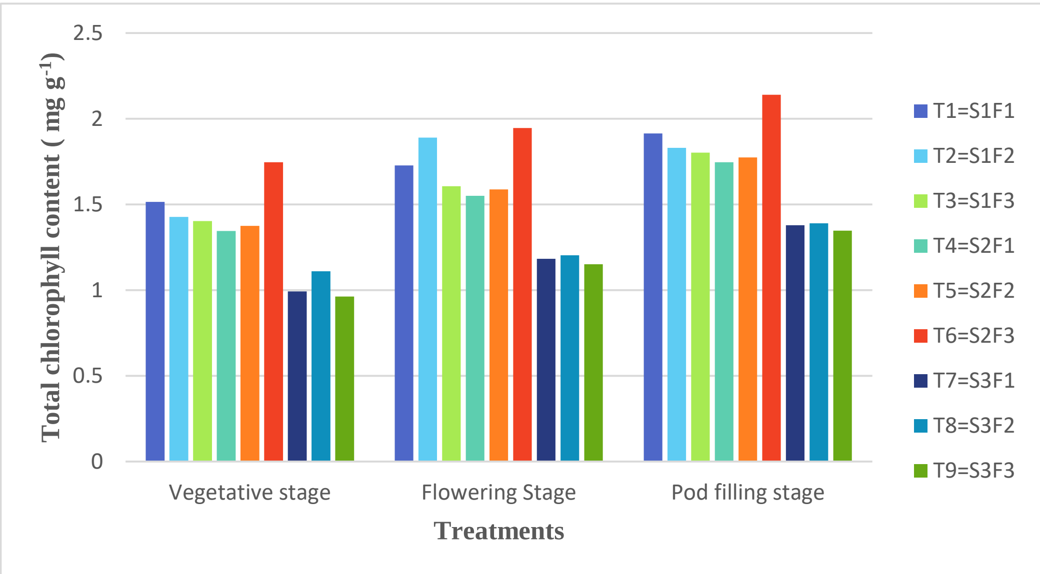

Fig. 10. Effect of different shade levels and moisture levels on total chlorophyll content of *C. tetragonoloba* (L.) Taub

5.1.1 EFFECT OF DIFFERENT SHADE LEVELS AND MOISTURE LEVELS ON TOTAL SOLUBLE PROTEIN

A significant variation was observed across the different treatments for the total soluble protein content. The findings of this study reveal that the combined effect of light and moisture stress is detrimental to the total soluble protein content in cluster bean. It was observed that there was significant reduction in the total soluble protein for treatments across all the growth stages studied, in comparison to control. The reduction was more pronounced in T3 (50% shade + 50% field capacity), where the total soluble protein was significantly reduced by up to 43.71% in comparison to control, T7 (open + 100% field capacity). The reduction was the least visible in T5 (25% shade + 75% field capacity), whereby 13.62% decrease in the total soluble protein was observed. Light stress in particular was the reason for the substantial reduction, with shade levels of S1 (50% shade) and S2 (25% shade) leading to a decrease of 25.24% and 12.58% respectively, when compared to control, S3 (open). The individual effect of moisture stress on the total soluble protein was relatively mild in comparison to light stress. F2 (75% field capacity) resulted in a slight increase of 1.05% whereas F3 (50% field capacity) reduced the total soluble protein by 6.12% compared to control, F1 (100% field capacity).

The results obtained in the study were similar to that of Garggi (2014), who found that the highest total soluble protein content was present in the *Amaranthus spinosus* plants kept under open condition. It was noted that water deficit stress resulted in a decrease in the total soluble protein content in plants. Those plants which were exposed to 50% shade treatment had the lowest total soluble proteins when coupled with severe water deficit stress of 50% field capacity. Hence, the interaction effect of light and moisture stress was more severe in relation to their individual effects.

The exposure to severe shading impairs the accumulation of total soluble proteins by the plants. The decline in the total soluble protein might have been associated with the shade related down regulation of the amount or activity of Rubisco (Evans and Seemann, 1989). Rubisco enzyme accounts for nearly 80 per cent of the soluble proteins in leaves of most of the plants (Joseph *et al.,* 1981). Diethelm and Shibles (1989) opined that the Rubisco content per unit leaf area was positively assosciated with that of soluble protein content of the leaf. The synthesis of proteins might have been seriously impaired due to the oxidative damage caused by the reactive oxygen species generated under stress conditions which hampers the functionality of many enzymes such as Rubisco.

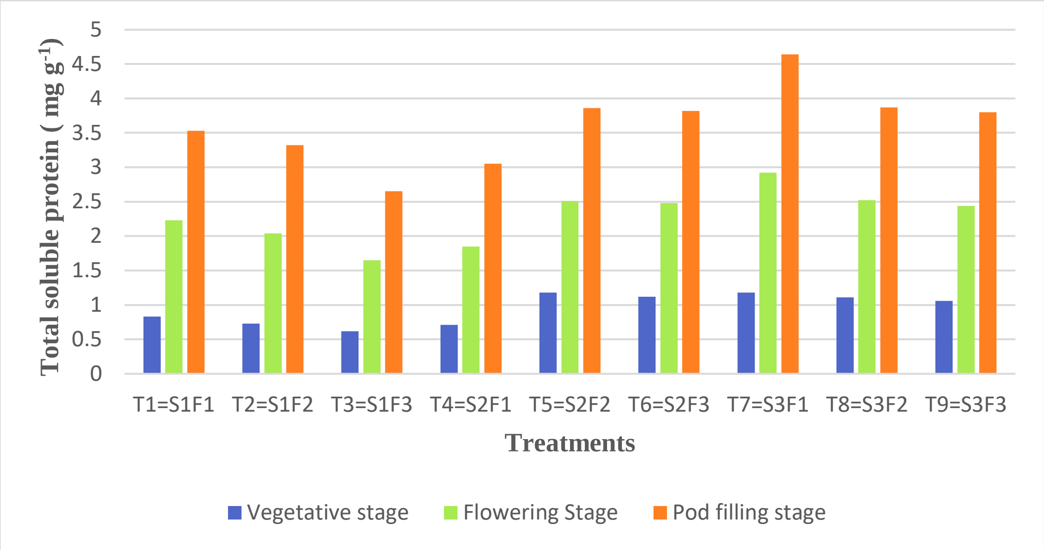

Fig. 11. Effect of different shade levels and moisture levels on total soluble protein of *Cyamopsis tetragonoloba* (L.) Taub.

Summary

6. SUMMARY

The research work entitled "Physiological and biochemical studies in cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] as influenced by light and moisture stress" was carried out with an objective to study light and moisture stress induced physiological and biochemical changes in cluster bean. The experiment was carried out during 2018-2020 at College of Agriculture, Vellayani, Thiruvananthapuram. Prominent findings of the experiment are summarized below.

A field study was carried out with the cluster bean variety 'KAU Suruchi' in Factorial Randomized Block Design (FRBD). It involved a combination of three levels of light stress (different shade levels) and three levels of moisture stress (different field capacity levels) which is inclusive of a control also, with four replications. The nine treatments were T1, T2 and T3 (50% shade in combination with 100%, 75% and 50% field capacities respectively), T4, T5 and T6 (25% shade in combination with 100%, 75% and 50% field capacities respectively), T7, T8 and T9 (open condition in combination with 100%, 75% and 50% field capacities respectively). The observations were taken at three different critical stages of the crop *viz.* vegetative stage, flowering stage and pod filling stage. Different shade levels were provided by using high-density polyethylene nets with differential light transmission to maintain 50 % and 25% shade and no shade net for open condition. Whereas for imposing moisture stress, three irrigation regimes based on gravimetric estimations were followed to maintain the field capacity levels of 100%, 75% and 50%.The treatment T7 (open condition + 100% field capacity) served as the control for the experiment.

The different shade levels and field capacity levels had a significant effect on the physiological parameters *viz.* plant height, shoot weight, crop growth rate, relative growth rate, photosynthetic rate and transpiration rate across all the critical growth stages. It was observed that the no. of primary branches plant^{-1} was significantly affected by the combined light and moisture stress only in the flowering stage. The different shade levels and field capacity levels significantly affected the root weight in the flowering and vegetative stages, whereas for root: shoot ratio the significant effect was visible only in the vegetative stage. It was also noteworthy that the different field capacity levels were statistically insignificant for the no. of primary branches plant^{-1} in the pod filling stage.

Plant height showed significantly higher response in all the critical growth stages, in the treatment provided with 25% shade and 50% field capacity (T6), improving the plant height by 14.1% in comparison to the control treatment, T7 (open + 100% field capacity). Severe shade level of 50% (S1) proved to be down regulating the plant height whereas decreasing field capacities of 75% and 50% resulted in smaller plants

The maximum number of primary branches plant^{-1} was recorded in the control, T7 (open + 100% field capacity) across all the growth stages studied. In the flowering stage, number of primary branches plant^{-1} were significantly reduced by 47.06% in the treatment T3 (50% shade + 50% field capacity), compared to that of control, T7. Overall, shading and water deficit, individually as well as in combination reduce the number of primary branches per plant.

In vegetative stage, the root dry weight was significantly up regulated for T3 (50% shade + 50% field capacity) to an extent of 46.88% in comparison to control, T7. In the flowering stage, the root dry weight was up regulated for T6 (25% shade + 50% field capacity) by 43.18% in comparison to control, T7.Light stress and moisture stress individually had a positive impact on the root dry weight across all the growth stages. Light stress levels of S1 (50% shade) and S2 (25% shade) improved the root dry weight by 29.16% and 18.41% respectively in comparison to control. Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) increased the root dry weight by 2.37% and 10.8% respectively, compared to control, F1 (100% field capacity).

It was found that the stresses in combination led to the significant decrease in shoot dry weight for the different treatments, in comparison to the control, T7 (open + 100% field capacity). It was found that the reduction was the most evident in statistically on-par treatments, T3 (50% shade + 50% field capacity) and T2 (50% shade + 75% field capacity), to an extent of 40.03% across all the growth stages. Light and moisture stress separately had a significant effect on the shoot dry weight across the different critical growth stages studied. Overall, shading and water deficit, individually as well as in combination reduced the number of primary branches plant^{-1}.

In the vegetative stage, significantly highest root: shoot ratio was observed in T3 (50% shade + 50% field capacity) and T2 (50% shade + 75% field capacity) which is an enormous increase of 200% and 163.64% respectively, over the control, T7 (open + 100% field capacity).Light stress had a positive impact on the root : shoot ratio, with shade levels, S1(50% shade) and S2 (25% shade) improving the root : shoot ratios by 120.65% and 80.07% respectively, over the control level, S3 (open). Moisture stress levels in terms of F2 (75% field capacity) and F3 (50% field capacity) enhanced the root: shoot ratio by 7.97% and 20.69% respectively, over the control level, F1 (100% field capacity).

It was found that the stresses in combination significantly decreased the CGR for the different treatments, in comparison to the control, T7 (open + 100% field capacity). It was observed that the reduction was the most apparent in treatment T3 (50% shade + 50% field capacity) to a degree of 52.68% across all the growth stages. Light and moisture stress distinctly had a significant effect on the CGR across the different critical growth stages studied. Light stress levels of S1 (50% shade) and S2 (25% shade) diminished the CGR by 47.87% and 39.73% respectively, compared to control, S3 (open). Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) decreased CGR by 6.05% and 8.19% respectively, in comparison to control, F1 (100% field capacity).

It was found that the stresses in combination led to a significant drop in the RGR for the different treatments, in comparison to the control, T7 (open + 100% field capacity). It was observed that the decline was the most obvious in treatment T3 (50% shade + 50% field capacity)), to a degree of 38.59% across all the growth stages. Light and moisture stress alone had a significant effect on the RGR across the different critical growth stages studied. Light stress levels of S1 (50% shade) and S2 (25% shade) lessened the RGR by 31.86% and 23.25% respectively, compared to control, S3 (open). Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) reduced the RGR by 5.06% and 7.64% respectively, in comparison to control, F1 (100% field capacity).

It was observed that the stresses in combination led to a significant decline in photosynthetic rate for the different treatments, in comparison to the control, T7 (open + 100% field capacity). It was noticed that the decline was the most evident in treatment T3 (50% shade + 50% field capacity), to a degree of 58.58% across all the growth stages. Light and moisture stress distinctly had a significant effect on the photosynthetic rate across the different critical growth stages studied. Light stress levels of S1 (50% shade) and S2 (25% shade) lowered the photosynthetic rate by 52.13% and 35.7% respectively, compared to control, S3 (open). Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) reduced the photosynthetic rate by 6.49% and 11.38% respectively, in comparison to control, F1 (100% field capacity).

It was observed that the stresses in combination led to a significant reduction in transpiration rate for the different treatments, in comparison to the control, T7 (open + 100% field capacity). It was noticed that the lowest transpiration rate was recorded in the treatment T3 (50% shade + 50% field capacity) to a degree of 48.43% across all the growth stages, compared to the control. Light and moisture stress distinctly had a significant effect on the transpiration rate across the different critical growth stages studied. Light stress levels of S1 (50% shade) and S2 (25% shade) lessened the transpiration rate by 39.64% and 23.76% respectively, compared to control, S3 (open). Moisture stress levels of F2 (75% field capacity) and F3 (50% field capacity) reduced the transpiration rate by 4.43% and 8.86% respectively, in comparison to control, F1 (100% field capacity).

The different shade levels and field capacity levels had a significant effect on all the biochemical parameters under the study *viz.* chlorophyll a, chlorophyll b, chlorophyll a: b ratio, total chlorophyll content and total soluble protein across all the growth stages.

Under the influence of both light and moisture stress, chlorophyll a content was enhanced by about 48.11% in T6 (25% shade+ 50% field capacity) As far as the individual effects are concerned, light stress *i.e.* S1 (50% shade) and S2 (25% shade) resulted in increments of 28.37% and 30.25% in the chlorophyll a content, compared to that of control, S3 (open). Moisture stress in terms of F2 (75% field capacity) and F3 (50% field capacity) however contributed to only 2.64% and 6.20% increase in the chlorophyll a content in comparison to control, F1 (100% field capacity).

The combination of light and moisture stress, improved chlorophyll b content by an enormous 157.36% in T6 (25% shade+ 50% field capacity), compared to the control, T7 (open + 100% field capacity).However, under open conditions, chlorophyll b content declined in T8 (open + 75% field capacity) and T9 (open + 50% field capacity) by 3.44% and 30.59% respectively. As far as the individual effects are concerned, light stress *i.e.* S1 (50% shade) and S2 (25% shade) resulted in massive increments of 166.09% and 129.16% in the chlorophyll b content, compared to that of control, S3 (open). Moisture stress in terms of F2 (75% field capacity) and F3 (50% field capacity) however led to a slight decrease of 2.26% and 14.37% increase in the chlorophyll b content in comparison to control, F1 (100% field capacity).

Under the influence of both light and moisture stress, chlorophyll a: b ratio was found to decrease significantly in comparison to the control, T7 (open + 100% field capacity). Severe decline in the chlorophyll a: b ratio was observed in T3 (50% shade + 50% field capacity), by about 59.81%. However under open conditions, the chlorophyll a: b ratio was considerably increased by up to 53.06% in T9 (open + 50% field capacity). The individual effects of the light and moisture stress on the chlorophyll a: b ratio were drastically different to each other. Light stress proved to be detrimental for the chlorophyll a: b ratio with S1 (50% shade) and S2 (25% shade) down regulating the ratio by 62.46% and 54.19% respectively in comparison to the control, S3 (open). However, moisture stress had a positive impact on the chlorophyll a: b ratio with F2 (75% field capacity) and F3 (50% field capacity) enhancing the chlorophyll a: b ratio by 6.84% and 17.63% respectively in comparison to control, F1 (100% field capacity).

The total chlorophyll content was enhanced under all treatments with a significant increase of up to 64.18% total chlorophyll content in treatment T6 (25% shade+ 50% field capacity).The combined influence of light and moisture stress is favourable for the enhancement of the total chlorophyll content in Cluster bean. However, in the present study, the detrimental effect of moisture stress on the chlorophyll content could be observed through about 2.64% reduction in mean total chlorophyll content, for treatment T9 (open+50% field capacity). The total chlorophyll content was also increased under the individual stresses but the increment was more evident under light stress than that of moisture stress. Light stress *i.e.* S1 (50% shade) and S2 (25% shade) led to an increase of 41.04% and 41.97% respectively in the total chlorophyll content; whereas moisture stress in terms of F2 and F3 led to a meagre increment of 1.75% and 5.66% in the total chlorophyll content.

It was observed that there was significant reduction in the total soluble protein for treatments across all the growth stages studied, in comparison to control. The reduction was more pronounced in T3 (50% shade + 50% field capacity), where the total soluble protein was significantly reduced by up to 43.71% in comparison to control, T7 (open + 100% field capacity). The reduction was the least visible in T5 (25% shade + 75% field capacity), whereby 13.62% decrease in the total soluble protein was observed. Light stress in particular was the reason for the substantial reduction, with shade levels of S1 (50% shade) and S2 (25% shade) leading to a decrease of 25.24% and 12.58% respectively, when compared to control, S3 (open). The individual effect of moisture stress on the total soluble protein was relatively mild in comparison to light stress. F2 (75% field capacity) resulted in a slight increase of 1.05% whereas F3 (50% field capacity) reduced the total soluble protein by 6.12% compared to control, F1 (100% field capacity).

Considering the physiological, and biochemical characters, treatment T6 (25% shade + 50% field capacity) is favourable for the tolerance of cluster bean to the light and moisture stress, in view of the enhancement of plant height and chlorophyll content and the comparatively better performance than other stress combinations in comprehending the stress combination. However, the severest stress combination of treatment T3 (50% shade + 50% field capacity) enhanced the root: shoot ratio and lowered the transpiration rate the most, which is a positive aspect in terms of stress tolerance. In short, cluster bean is capable of tolerating light and moisture stress levels up to 50% shade and 50% field capacity respectively.

Future line of work

- The interaction effect of other abiotic stresses could be studied
- Stress induced secondary metabolite production could be assessed
- Molecular studies to enhance the development of stress tolerant genotypes

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PHYSIOLOGICAL AND BIOCHEMICAL STUDIES IN CLUSTER BEAN [*Cyamopsis tetragonoloba* (L.) Taub.] AS INFLUENCED BY LIGHT AND MOISTURE STRESS

By

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ABSTRACT

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8. ABSTRACT

The programme entitled "Physiological and biochemical studies in cluster bean [*Cyamopsistetragonoloba*(L.) Taub.] as influenced by light and moisture stress" was carried out at the Department of Plant Physiology, College of Agriculture, Vellayani, during 2019-2020. The objective of the programme was to study the light and moisture stress induced physiological and biochemical changes in cluster bean.

A field study was carried out with the cluster bean variety 'KAU Suruchi' in Factorial Randomized Block Design (FRBD). It involved a combination of three levels of light stress (different shade levels) and three levels of moisture stress (different field capacity levels) which is inclusive of a control also, with four replications .The observations were taken at three different critical stages of the crop *viz.*vegetative stage, flowering stage and pod filling stage. Different shade levels were provided by using high-density polyethylene nets with differential light transmission to maintain 50 % and 25% shade and no shade net for open condition. Whereas for imposing moisture stress, three irrigation regimes based on gravimetric estimations were followed to maintain the field capacity levels of 100%, 75% and 50%.The treatment (open condition + 100% field capacity) served as the control (T7) for the experiment.

Different growth parameters such as plant height, number of primary branches per plant, root dry weight, shoot dry weight, root: shoot ratio, crop growth rate and relative growth rate were studied. It was found that only root dry weight and root: shoot ratio increased under combined light and moisture stress in comparison to control . However, plant height was found to increase under the treatments T1 (50% shade + 100% field capacity), T4 (25% shade + 100% field capacity), T5 (25% shade + 75% field capacity) and T6 (25% shade + 50% field capacity) compared to the control.

The physiological parameters studied *viz.* transpiration rate and photosynthetic rate were found to decrease under the influence of both light and moisture stress conditions compared to the control.

Among the biochemical parameters studied, total chlorophyll content was found to increase under the influence of both the stresses with the highest value (1.747 mg g^{-1}, 1.947 mg g^{-1} and 2.141 mg g^{-1}) across the three growth stages except that of treatment T9 (open + 50% field capacity).The chlorophyll 'a'content was found to increase across all the treatments compared to the control. However chlorophyll 'b' content was found higher under shaded condition. But the chlorophyll a: b ratio was found to be higher only under open conditions. The total soluble protein content showed a decrease under the combined abiotic stresses.

Overall it was observed that under the combined influence of light and moisture stress, physiological parameters such as plant height(T1,T4,T5 and T6), root weight and root: shoot ratio were up regulated whereas number of primary branches per plant, shoot weight, crop growth rate, relative growth rate, photosynthetic rate and transpiration rate were down regulated. As far as biochemical characters are concerned chlorophyll 'a', chlorophyll 'b' (T1 to T6), chlorophyll a: b ratio (T8 and T9) and total chlorophyll content (T1 to T8) improved, whereas the total soluble protein was found to decrease across all treatments compared to control.

Considering the physiological, and biochemical characters, treatment T6 (25% shade + 50% field capacity) is favourable for the tolerance of cluster bean to the light and moisture stress, in view of the enhancement of plant height and chlorophyll content and the comparatively better performance than other stress combinations in comprehending the stress combination. However, the severest stress combination of treatment T3 (50% shade + 50% field capacity) enhanced the root: shoot ratio and lowered the transpiration rate the most, which is a positive aspect in terms of stress tolerance. In short, cluster bean is capable of tolerating light and moisture stress levels up to 50% shade and 50% field capacity respectively.