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Influence of Water Stress and Rhizobial Inoculation on the Accumulation of Chlorophyll in *Phaseolus vulgaris* (L.) Cultivars

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EVT, KMM and PAN designed the study. Author EVT conducted both field and screen house trials and performed statistical analysis with supervision from authors KMM and PAN. Author EVT wrote the first draft of this manuscript. Authors KMM and PAN edited the manuscript. All authors read and approved the manuscript.

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ABSTRACT

Aims: To assess the effect of water stress periods and rhizobial inoculation in five (5) *P. vulgaris* (L.) cultivars.
Study Design: The experiment was designed in split-split plot and replicated 3 (three) times.
Place and Duration of Study: The field experiment was carried out for two consecutive seasons in the year 2014 and 2015, whereas, the screen house experiment was planted in a single season in the year 2016 at the Agricultural Seed Agency (ASA) farm in Arusha-Tanzania.
Methodology: The experiment consisted of 2 levels of rhizobia (with and without inoculation), two stress levels (with and without water stress) and five cultivars of *P. vulgaris* (L.) (*KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line* and *JESCA*). The stress period of 10 days were imposed at vegetative and flowering stages of plant growth by not irrigating. Chlorophyll was extracted using dimethyl sulphoxide (DMSO). Absorbance values were read at 645 nm and 663 nm by 2800 UV/Vis Spectrophotometer.
Results: Results indicated that leaf chlorophyll content was higher in rhizobial inoculated and non-

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stressed water treatments. Leaf chlorophyll content was significantly higher in varieties 3(*F9 Kidney Selection*) and 2(*KAT B1*) as compared with varieties 1(*KAT B9*), 4(*F8 Drought line*) and 5(*JESCA*). Significant interactions were observed between rhizobial inoculation x water stress and bean varieties.

Conclusion: Rhizobial inoculation and adequate water supply significantly improved leaf chlorophyll content in the tested cultivars.

Keywords: P. vulgaris (L.); water stress; rhizobial inoculation; chlorophyll.

1. INTRODUCTION

Light is the environmental factor that has most influence on growth and yield quantity and quality of crops, however low light intensity lowers the rate of photosynthesis [1]. Chlorophyll is the main chloroplast component for photosynthesis and substantial chlorophyll content has a constructive association with photosynthetic rate [2]. From physiological phenomena, leaf chlorophyll content is a unique entity with its own significant interest in plant [3]. Water stress is a serious threat to agriculture as it affects growth and plant pigments such as chlorophyll in different plant species. However, water stress tolerance mechanism varies significantly in different plant species. Changes in photosynthetic pigments are of chief importance to water stress and tolerance [4]. Under condition of moisture stress in soil, the rate of CO_2 fixation is reduced along with photosynthetic rate resulting in less assimilate production for growth and yields in plants [3]. A study by Ommen et al. [5] indicated that, moisture stress slow down photosynthesis of plants and cause changes in chlorophyll content by affecting chlorophyll components and by damaging the plant photosynthetic apparatus. The decreases in chlorophyll under this condition are mainly the result of destruction of chloroplasts caused by reactive oxygen species (ROS) [6]. It has been reported that chlorophyll *a* and *b* are susceptible to soil water deficit [7-9]. Studies have revealed that water deficit results in negative impact in plants as majority of chlorophyll are lost (2, 3 & 5). Normally, these losses occur in mesophyll cells than in the bundle sheath [10]. Study by Baroowa and Gogoi [11] in Black gram and Green gram indicated that chlorophyll content decreased with the increasing water stress and hence confirming that photosynthetic pigments were sensitive to water stress conditions. Report by, Massacci et al. [12] shows reduction in chlorophyll content in drought stressed cotton. Santos et al. [4] found that in moderate water stress conditions, the net photosynthetic rate decreased in common beans. Another study in sunflower plants also shows a significant decrease in chlorophyll content at higher water deficits [13]. The photosynthetic rate of higher plants is known to be reduced as the relative water content and leaf water potential decreases [14]. Abu-Muriefah, [15] showed that water stress in common bean (*P. vulgaris* L.) impairs photosynthetic pigments in plant tissues, mainly shoot. It has been further reported that, reduction in leaf chlorophyll content under drought stress might be due to the excessive swelling of chloroplast membranes and distortion of the lamellae vesiculation in the plant tissues [16,17]. It can be established that the decline in photosynthesis observed under water stress could be attributed by stomatal factors (i.e. stomatal and non-stomatal limitations); of which the concentration of CO_2 in chloroplasts decreases because of a reduction in stomatal conductance [7,18-20].

Apart from water, nitrogen is the major component of the chlorophyll molecules and plays an essential function in photosynthesis process, protein formation and many enzymatic processes in plants [21-24]. With N_2 deficient soils, the use of nitrogenous fertilizers and/or suitable rhizobial strains might improve legume growth by enhancing photosynthesis and chlorophyll formation. Study by Anjum et al. [25] in Mungbean showed that beneficial rhizobia bacteria influence the physiological growth conditions by providing N through fixation thus increasing chlorophyll contents in leaves. However, N_2 deficiency give a negative response in plants by showing symptoms of yellowing which demonstrate chlorophyll deterioration has occurred in plants and therefore cause reduction in photosynthesis rate [26]. It is established that soil moisture deficit has a distinct effect on N_2 fixation as it affects nodule formation, growth and photosynthesis activities. However, appropriate-competitive nodulating strains and suitable tolerant host legume varieties may play a significant role in the photosynthesis process and chlorophyll formation under stressed environment [19]. Study done by Tajini et al. [27] shows reduction in chlorophyll concentration

under water deficit in common beans using two strains of Rhizobia. Therefore, the objective of this study is to investigate the influence of water stress and rhizobial inoculation on the accumulation of chlorophyll content in selected *P. vulgaris* (L.) cultivars.

2. MATERIALS AND METHODS

2.1 Description of Site Location

The trial was conducted at Agricultural Seed Agency (ASA) farm in Arusha, located at Latitude 3°18′S and Longitude 36°38′06.29″E. ASA receives mean annual rainfall of 819 mm, mean temperature of 19.15℃ with relative humidity of about 94% and altitude of 1520 masl. The field trial was carried out during dry season of January, to March 2014 and January, to March, 2015 while the screen house experiment was carried out from mid January to March, 2016 under irrigation.

2.2 Experimental Design and Treatment Application

The experiment was designed in split, split plot with 3 replications. The plot size was 3 by 4m. The field experimental treatments consisted of 2 levels of Rhizobia (with and without inoculation) as the main factor followed by imposing of stress (sub factor) in vegetative and flowering stages of plant growth. Five cultivars of *P. vulgaris* (L.) (*KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line and JESCA*) were assigned to sub-sub plots. These cultivars were selected based on the fact that Varieties *F8 Drought Line, KAT B1* performed well in preliminary screening studies for drought tolerance [28,29]. Bean variety J*ESCA* was included because in a potted study, it showed moderate tolerance to salinity [30]. Cultivars *F9 Kidney Selection*, *F8 Drought Line* and *KAT B9* have good adaptability in some production areas in the medium altitude zone of Tanzania. They have earned good approval by beneficiaries and are early maturing, drought tolerant, resistant to major diseases and have sufficient yielding [28,29]. The common bean seeds were sown at a spacing of 50 cm by 20 cm, making a plant population density of 200,000 plants per hectare. The BIOFIX legume inoculants were obtained from *MEA* Company Nairobi-Kenya, sold under license from the University of Nairobi. Common bean seeds lines and/or varieties *KAT B9, KAT B1, F9 Kidney Selection, F8 Drought line and JESCA* were obtained from the breeding unit based at Selian Agricultural Research Institute (SARI), Arusha, Tanzania. Land for field experiment was cleared and all the necessary practices like ploughing and harrowing were done before planting. Moreover, in the screen house experiment, wooden box technique was used to establish the experiment. This was done by collecting the same soil used at field experiment and beans were planted using the protocol developed by [31] with some modifications. Common bean seeds were thoroughly mixed with *R. leguminosarum* inoculants to supply (10^9 cells/g seed), following procedure stipulated by products manufacturer. To avoid contamination, all non-inoculated seeds were sown first, followed by inoculated seeds. Three seeds were sown and thinned to two plants per hill after full plant establishment. Stress period of 10 days were imposed at vegetative and flowering stages of plant growth by not irrigating.

2.3 Plant Harvest and Sample Preparation

Plant leaf samples from field and glasshouse experiments were collected for chlorophyll analysis. In the field experiment, 10 plants were randomly sampled from the middle rows of each plot while in the glasshouse experiment two plants from each pot were sampled. The fresh plant leaf samples from each of the growth stages (i.e. vegetative and flowering) were collected from the third young leaf from the top and kept in ice container to maintain their freshness for chlorophyll analysis.

2.4 Determination of Chlorophyll (Chl) Contents in Plant Leaves

Extraction of chlorophyll concentrations by dimethylsulphoxide (DMSO) was done as described in Hiscox and Israelstam [32]. A third of the plants leaves from the tip were collected from each plot. A hundred (100 mg) of the middle portion of fresh leaf slices was placed in a 15 ml vial containing 7 ml dimethylsulphoxide (DMSO) and incubated at 4℃ for 72 hours. After the incubation, the extract was diluted to 10 ml with DMSO. The DMSO technique extracts chlorophyll from shoot tissue without grinding or maceration [32]. A 3 ml sample of chlorophyll extract was then transferred into curvets for absorbance determination. A spectrophotometer (2800 UV/Vis Spectrophotometer) was used to determine absorbance values at 645 and 663 (nm), which was then used by Arnon [33] to

determine Leaf Chlorophyll *'a'*, Leaf Chlorophyll '*b'* and Total Leaf Chlorophyll expressed as mgL^{-1}.

The equation is expressed as follows;

Chlorophyll '*a'* = [(12.7 X OD at 663) – (2.69 X OD at 645)]
Chlorophyll '*b'* = [(22.9 X OD at 645) – (4.68 X OD at 663)]
Chlorophyll Total = [(20.2 X OD at 645) + (8.02 X OD at 663)]

Where by OD = Optical density which present the absorption in 645 and 663 nm.

2.5 Statistical Analysis

A 3-way ANOVA was used to analyze the data collected. The analysis was done using STATISTICA software program of 2013. Fisher's least significant difference was used to compare treatment means at $P = 0.05$ [34].

3. RESULTS

3.1 Effect of Inoculation with *R. leguminosarum bv. phaseoli* and Stress Period in Chlorophyll '*a'*, '*b'* and Total Chlorophyll in Selected *P. vulgaris* (L.) Varieties

Results in Tables 1 and 2 showed that water stress and rhizobial inoculation significantly influenced chlorophyll '*a'*, '*b'* and total chlorophyll content in both field and screen house experiment. Rhizobial inoculation significantly increased chlorophyll '*a'* by 17%, '*b'* by 30% and total chlorophyll content by 20% in vegetative stage and 18% in flowering stage in season one (Table 1). Significant increase in chlorophyll *'a'*, '*b'* and total chlorophyll via rhizobial inoculation was also observed in season two by 47, 70 and 42% in vegetative and 18% for chlorophyll '*b'* and 15% for total chlorophyll in flowering stage respectively (Table 1). In season one, water stress period significantly increased the chlorophyll '*a'* at flowering stage by 14 % over the control (Table 1). In season two, water stress periods significantly influenced chlorophyll '*a'*, '*b'* and total chlorophyll content at vegetative stage by 27, 10 and 39% and at flowering stage by 47, 57 and 38% respectively (Table 1). However, for screen house experiment, water stress significantly affected chlorophyll '*b'* and total chlorophyll at flowering stage by 5 and 10% respectively (Table 2). In general term, variety 2 and 3 proved to have significantly greater chlorophyll content under field and screen house experiment in both seasons (Tables 1 and 2).

3.2 Interactive Effects of Inoculation with *R. leguminosarum bv. phaseoli* and Stress Period on Chlorophyll '*a'*, '*b'* and Total Chlorophyll in Selected *P. vulgaris* (L.) varieties

There was a significant interaction between *R. leguminosarum bv. phaseoli* and stress period/levels in chlorophyll '*a'*, chlorophyll '*b'* and total chlorophyll content in season one at vegetative and flowering stages together with total chlorophyll in season two at vegetative stage respectively (Figs. 1-4 and 6). *R. leguminosarum bv. phaseoli* treatment without water stress resulted into increased levels of chlorophyll '*a'*, '*b'* and total chlorophyll (mgL^{-1}) content compared with treatments with no *R. leguminosarum bv. phaseoli* inoculants with water stress (Figs. 1-4 and 6). The trend of interaction in chlorophyll '*b'* was also observed between *R. leguminosarum bv. phaseoli* and bean varieties at vegetative stage in season two (Fig. 5). Significant interaction in chlorophyll '*a*' content was also observed between water stress and bean varieties in the second season at flowering stage (Fig. 7). Under all the interactions mentioned, rhizobial inoculation and the control (No stress treatment S_I) increased chlorophyll '*a'*, '*b'* and total chlorophyll content in both seasons in this study (Figs. 1-7).

4. DISCUSSION

Nitrogen is a primary nutrient which plays most important roles in legumes and is a major constituent of chlorophyll which is the most essential pigment needed for photosynthesis and amino acids in plants [26]. In this study, rhizobial inoculation was reported to increase chlorophyll content of *P. vulgaris* (L.) cultivars compared with un-inoculated treatments. The increased chlorophyll in inoculated treatments may be due to improved plant growth due to enhanced photosynthesis and hence chlorophyll formation. In similar studies, Lalitha and Santhaguru, [35] showed increased chlorophyll content in inoculated plants with *Rhizobium*. In relation to this study, it has been reported that rhizobial inoculation may influence the physiological growth condition of leguminous plants by increasing leaf photosynthesis [24,36] and Chl

contents in the leaves [26,37–40]. Results from this study suggest that the supplied *R. leguminosarum bv. phaseoli* promoted the plant growth through a mechanism which increased Chl synthesis and photosynthetic rate in plants.

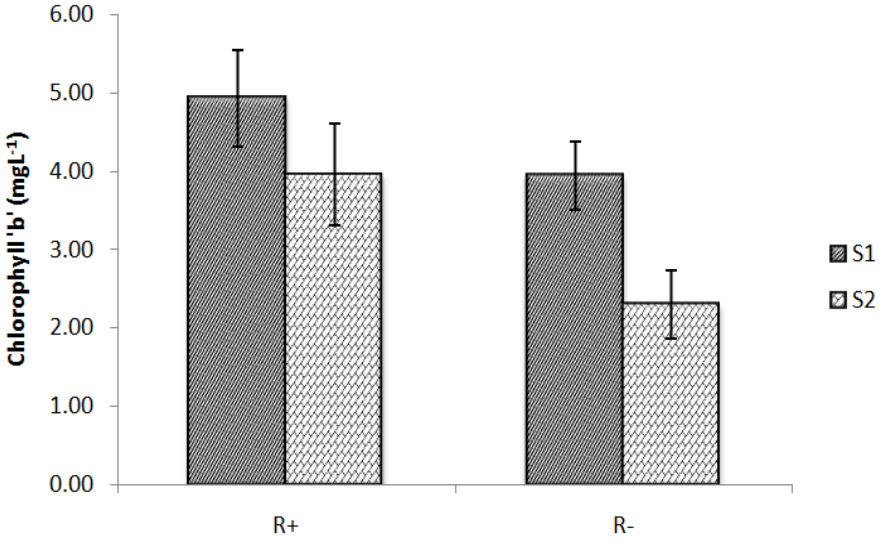

Fig. 1. Interactive effects of *R. leguminoserum bv. phaseoli* and stress level on chlorophyll '*b*' in season (1) field experiment under vegetative stage (+R-: With *R. leguminoserum bv. phaseoli*, -R-: Without *R. leguminoserum bv. phaseoli*, S1-: Control, S2-: Water stress at vegetative stage)

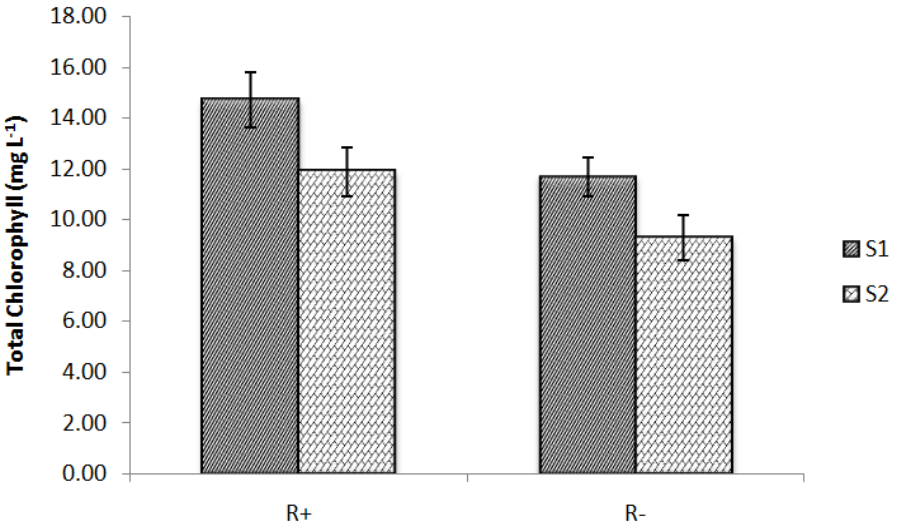

Fig. 2. Interactive effects of *R. leguminoserum bv. phaseoli* and stress level on total chlorophyll content in season (1) field experiment under vegetative stage (+R-: With *R. leguminoserum bv. phaseoli*, -R-: Without *R. leguminoserum*. *bv. phaseoli*, S1-: Control, S2-: Water stress at vegetative stage)

Table 1. Effect of with and without *R. leguminosarum,* stress period, and five (5) *P. vulgaris* (L.) in the chlorophyll '*a*', chlorophyll '*b*' and total chlorophyll on plant leaves as measured on field experiment in two consecutive seasons

Growth phases	1st season						2nd season					
	Vegetative			Flowering			Vegetative			Flowering		
Treatments *inoculation*	**Chl a(mgL^{-1})**	**Chl b(mgL^{-1})**	**Total Chl (mgL^{-1})**	**Chl a(mgL^{-1})**	**Chl b(mgL^{-1})**	**Total Chl (mgL^{-1})**	**Chl a(mgL^{-1})**	**Chl b(mgL^{-1})**	**Total Chl (mg^{-1})**	**Chl a(mgL^{-1})**	**Chl b(mgL^{-1})**	**Total Chl (mg^{-1})**
R+	8.34±0.45a	4.43±0.38a	13.18±0.71a	9.99±0.76a	6.06±0.59a	14.81±1.07a	11.29±0.73a	8.38±0.39a	15.98±0. 98a	11.65±0.95a	7.28±0.65a	20.20±1.14a
R-	6.91±0.38b	3.12±0.41b	10.58±0.69b	8.24±0.58b	5.70±0.54a	14.07±0.90a	6.04±0.25b	2.54±0.19b	9.21±0.54 b	12.94±0.99a	5.94±0.63b	17.25±1.02b
Stress levels												
S_1	7.73±0.43a	3.61±0.44a	11.99±0.86a	9.82±0.80a	6.12± 0.62a	14.90±1.08a	10.00±0.83a	5.75±0.68a	15.66±0.93 a	16.09±0.90a	9.26±0.47a	23.06±0.93a
S_2/S_3	7.52±0.45a	3.95±0.39a	11.77±0.61a	8.42±0.55b	5.65± 0.51a	13.98±0.88a	7.33±0.50b	5.17±0.55b	9.53±0.72b	8.49±0.31b	3.96±0.38b	14.38±0.58b
Varieties												
V_1	7.44±0.44b	3.23±0.34c	12.77±1.08b	8.34±0.31bc	6.44 ±0.52b	15.04±0.85b	8.67±1.19a	5.43±0.84b	12.80±1.51 ab	12.19±1.27bc	6.99±0.98ab	18.51±1.24b
V_2	8.68±0.59b	4.70±0.64b	13.58±0.59ab	9.46±0.41b	7.98 ±0.50a	16.72±0.72b	9.84±1.36a	6.68±1.09a	14.19±1.81 a	16.03±2.05a	8.40±1.03a	23.39±2.13a
V_3	10.41±0.49a	6.48±0.50a	15.31±1.03a	13.92±1.52a	8.9 2±0.67a	20.39±1.33a	9.53±1.32a	6.81±1.13a	14.33±1.7 9a	13.62±1.62b	7.51±0.99a	20.46±1.53b
V_4	5.82±0.43c	2.25±0.42c	9.51±0.99c	6.97±0.50c	2.25±0 .29d	9.76±1.30c	7.51±0.91a	4.14±0.87c	10.79±1.37b	9 .62±0.85d	4.84±0.82c	16.06±1.30c
V_5	5.77±0.36c	2.24±0.38c	8.22±0.76c	6.89±0.70c	3.82±0 .57c	10.28±0.95c	7.79±0.90a	4.26±0.73c	10.87±1.30b	10.00±0.94cd	5.32±1.08bc	15.20±1.52c
3-Way Anova (F-Statistics)												
Rhz	12.55**	15.75***	14.36***	6.71*	0.75ns	0.53ns	51.35***	462.41***	89.69***	3.25ns	6.04*	18.96***
StrL	0.26ns	1.04ns	0.10ns	4.27*	1.27ns	0.82ns	13.27***	4.56*	73.69***	113.65***	94.74***	163.32***
Vrty	19.15***	24.19***	14.62***	14.51***	35.96***	15.58***	1.56ns	17.63***	4.63**	11.06***	6.04***	19.23***
Rhz*StrL	0.68ns	16.07***	16.84***	7.03*	9.17**	3.27ns	2.54ns	2.08ns	4.57*	0.40ns	0.82ns	1.41ns
Rhz*Vrty	0.89ns	1.09ns	0.19ns	0.33ns	1.59ns	0.10ns	0.23ns	3.56*	0.36ns	0.16ns	0.33ns	0.15ns
StrL*Vrty	0.40ns	1.44ns	0.87ns	0.29ns	1.04ns	0.37ns	0.13ns	0.87ns	0.38ns	3.42*	0.09ns	2.23ns
Rhz*StrL*Vrty	0.25ns	1.29ns	0.46ns	0.82ns	1.88ns	0.35ns	0.19ns	2.41ns	0.48ns	0.33ns	0.08ns	0.96ns

*+R: With R. leguminosarum, −R: Without R. leguminosarum; S_1: No water stress, S_2: Water stress at Vegetative Stage, S_3: Water stress at Flowering Stage; V_1= Variety 1 (KAT B9), V_2=Variety 2 (KAT B1), V_3=Variety 3 (F9 Kidney Selection), V_4=Variety 4 (F8 Drought Line), V_5=Variety 5 (JESCA). Values presented are means ± SE. *, **, *** = significant at P ≤ 0.05, at P ≤ 0.01, and at P ≤ 0.001 respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at P = 0.05*

Table 2. Effects of chlorophyll '*a*', chlorophyll '*b*' and total chlorophyll in five (5) *P. vulgaris* (L.) plant leaves as influenced by water stress periods and rhizobial inoculation on screen house experiment in a single season

Growth phases	**Vegetative**			**Flowering**		
Treatments *inoculation*	**Chlorophyll '*a*' (mgL^{-1})**	**Chlorophyll '*b*' (mgL^{-1})**	**Total Chlorophyll (mgL^{-1})**	**Chlorophyll '*a*'(mgL^{-1})**	**Chlorophyll '*b*' (mgL^{-1})**	**Total Chlorophyll (mgL^{-1})**
R+	15.88±0.90a	14.46±0.33a	30.33±1.22a	16.39±0.96a	14.84±0.44a	32.22±1.35a
R-	17.45±0.95a	15.30±0.54a	32.74±1.38a	16.57±0.86a	14.55±0.42a	30.77±1.33a
Stress levels						
S_1	17.81±1.03a	15.00±0.46a	32.81±1.46a	16.59±1.02a	15.04±0.45a	33.19±1.41a
S_2/S_3	15.52±0.78a	14.76±0.44a	30.26±1.11a	16.37±0.79a	14.36±0.41b	29.79±1.21b
Varieties						
V_1	16.67±1.18ab	14.87±0.45abc	31.53±1.60ab	13.51±0.34c	14.46±0.15c	30.32±0.68c
V_2	18.62±1.58a	15.99±0.75a	34.60±2.13a	18.63±0.66b	15.89±0.19b	35.81±0.59b
V_3	19.27±1.80a	15.85±0.84ab	35.11±2.60a	25.25±0.76a	18.33±0.28a	43.38±1.09a
V_4	14.31±0.86b	13.76±0.29c	28.06±1.13b	13.04±0.93c	12.21±0.57d	24.09±1.37d
V_5	14.46±1.42b	13.93±0.90bc	28.38±2.09b	11.96±0.74c	12.59±0.54d	23.87±1.11d
3-Way Anova (F-Statistics)						
Rhz	1.58ns	1.83ns	1.84ns	0.08ns	0.76ns	3.26ns
StrL	3.37ns	0.15ns	2.04ns	0.11ns	4.11*	17.73***
Vrty	2.68*	2.24ns	2.79*	56.99***	45.57***	84.13***
Rhz*StrL	0.12ns	2.33ns	0.60ns	0.13ns	0.98ns	0.19ns
Rhz*Vrty	0.35ns	0.22ns	0.25ns	0.32ns	2.30ns	1.12ns
StrL*Vrty	1.22ns	0.54ns	0.84ns	1.82ns	0.84ns	1.80ns
Rhz*StrL*Vrty	1.12ns	1.40ns	1.29ns	0.52ns	0.61ns	0.45ns

*+R: With R. leguminosarum; −R: Without R. leguminosarum, S_1: No water stress, S_2: Water stress at Vegetative Stage, S_3: Water stress at Flowering Stage. V_1= Variety 1 (KAT B9), V_2=Variety 2 (KAT B1), V_3=Variety 3 (F9 Kidney Selection), V_4=Variety 4 (F8 Drought Line), V_5=Variety 5 (JESCA). Values presented are means ± SE. *, *** = significant at P ≤ 0.05 and at P ≤ 0.001 respectively, ns = Not significant. Means followed by similar letter(s) in a given column are not significantly difference from each other at P = 0.05*

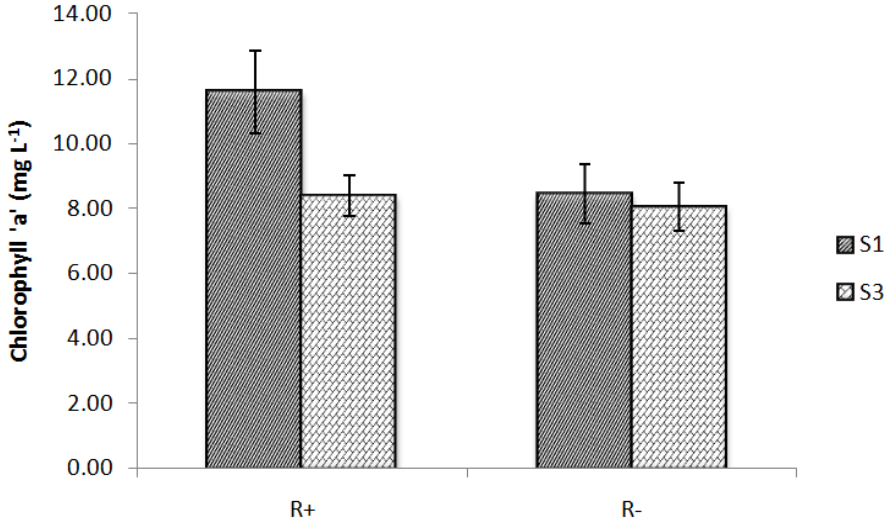

Fig. 3. Interactive effects of *R. leguminoserum bv. phaseoli* and stress level on chlorophyll '*a*' content in season (1) field experiment under flowering stage (+R-: With *R. leguminoserum bv. phaseoli*, -R-: Without *R. leguminoserum*. *bv. phaseoli*, S1-: Control, S3-: Water stress at flowering stage)

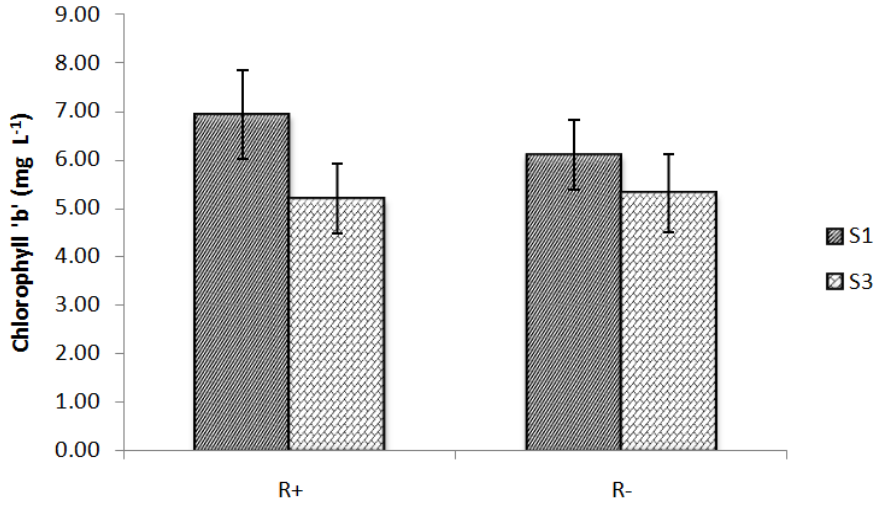

Fig. 4. Interactive effects of *R. leguminoserum bv. phaseoli* and stress level on chlorophyll '*b*' content in season (1) field experiment under flowering stage (+R-: With *R. leguminoserum bv. phaseoli*, -R-: Without *R. leguminoserum bv. phaseoli*., S1-:Control, S3-: Water stress at flowering stage)

In the present study, we assessed the effects of water stress in the accumulation of leaf chlorophyll content. Water stress caused a decrease in chlorophyll '*a*', '*b*' and total chlorophyll content of the common bean growth in fields and screen house experiments. The decreased or increased chlorophyll level during water stress at particular stages of plant growth has been reported in other plant species depending on the extent and severity of stress [41]. The reduction of chlorophyll under water stress condition might be contributed by moisture limitation which affected the photosynthesis process and hence the

chlorophyll formation. Cornic [42] reported that reduced water content in the plant results in the closure of the stomata and eventually reduces the rate of photosynthesis. Similarly, [11,43-49] showed that water stress damaged the photosynthetic machinery of the plants and reduced the chlorophyll content.

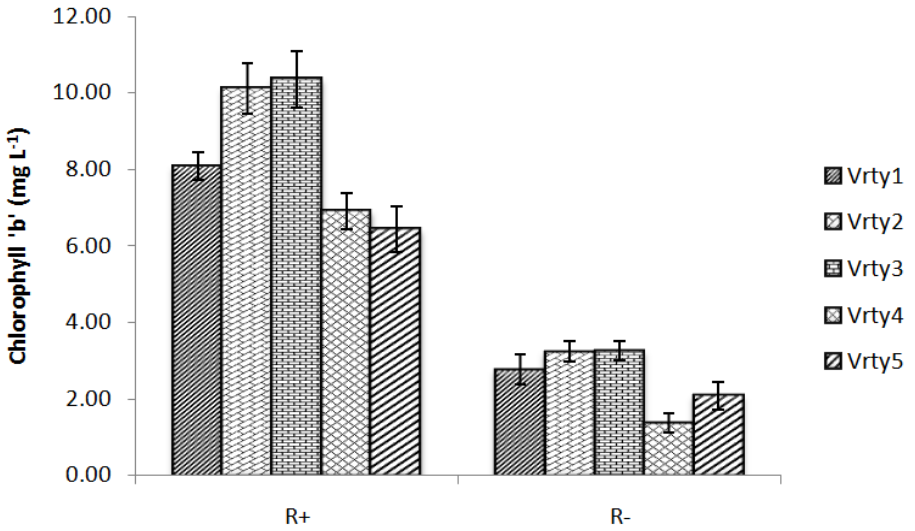

Fig. 5. Interactive effects of *R. leguminoserum bv. phaseoli* and five (5) *P. vulgaris* (L.) on chlorophyll '*b*' content in season (2) field experiment under vegetative stage, (R+: With *R. leguminoserum bv. phaseoli*, -R-: Without *R. leguminoserum bv. phaseoli*, Vrty1 -: KAT B9, Vrty2 -: KAT B1, Vrty3-: F9 Kidney Selection, Vrty 4 -: F8 Drought Line, Vrty5-: JESCA)

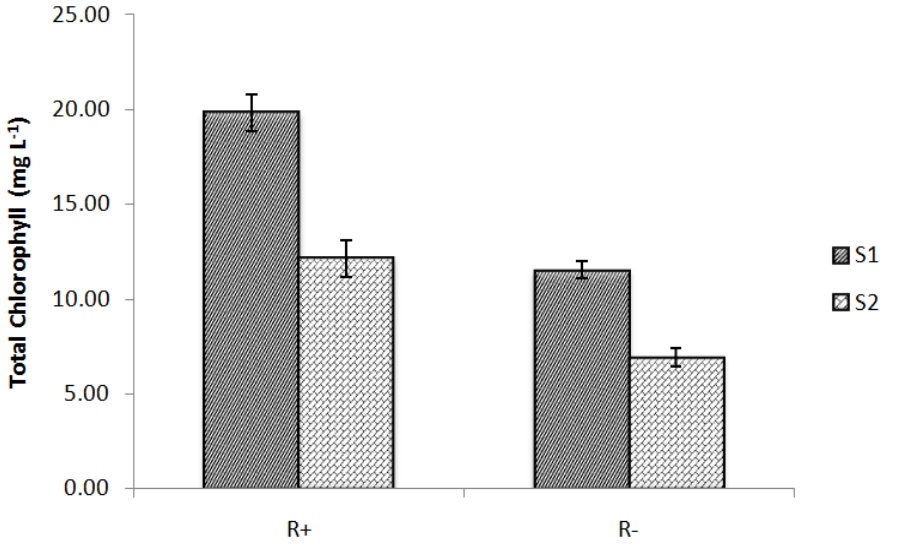

Fig. 6. Interactive effects of *R. leguminoserum bv. phaseoli* and stress level on chlorophyll total in season (2) field experiment under vegetative stage (+R-: With *R. leguminoserum bv. phaseoli*, -R-: Without *R. leguminoserum bv. phaseoli*, S1-: Control, S2-: Water stress at vegetative stage)

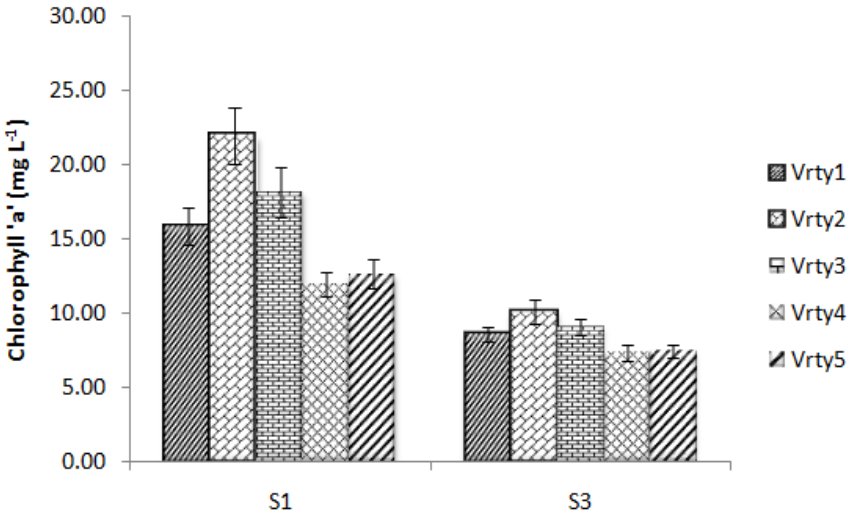

Fig. 7. Interactive effects of stress level and five (5) *P. vulgaris* (L.) on chlorophyll '*a*' content in season (2) field experiment under flowering stage, S1-: Control, S3-: Water stress at flowering stage, Vrty1-: KAT B9, Vrty2-: KAT B1, Vrty3-: F9 Kidney Selection, Vrty4-: F8 Drought Line, Vrty5-: JESCA)

In the present study, significant increase in chlorophyll '*a*', '*b*' and total chlorophyll content was seen in variety 3 (*F9 Kidney Selection*), and variety 2 (*KAT B1*) in both fields and screen house experiment as compared with varieties 1(*KAT B9*), 4(*F8 Drought line*) and 5(*JESCA*). The significance difference among the studied cultivars might be attributed by the genetic makeup in their chlorophyll metabolism. Moreover, the low chlorophyll content in varieties 1(*KAT B9*), 4(*F8 Drought line*) and 5(*JESCA*) could be attributed by damage to leaf pigments as a result of water deficit. The results of the current study propose that the photosynthesis potential of the tested varieties is different, and hence may affect some of the physiological functions of the plant. These results are in agreement with Nyachiro et al. [50] who reported a significant decrease in chlorophyll '*a*' and '*b*' in six *Triticum aestivum* cultivars. Similar study on common bean showed reduction in net photosynthetic rate and chlorophyll concentration as a result of water stress [4,51].

The significant interactive effects observed between water stress x Rhizobia x varieties in Chlorophyll '*a*', chlorophyll '*b*' and total chlorophyll is an indication that N which was supplied by N_2 fixation, enough moisture in the growth media and efficient cultivars are necessary in improving chlorophyll synthesis in *P. vulgaris* (L.).

5. CONCLUSION

In conclusion, rhizobial inoculation and adequate water supply significantly improved total leaf chlorophyll content at vegetative and flowering in season 2 and at flowering in glasshouse and field experiment. Furthermore, the varieties tested also differed significantly in their potential to accumulate chlorophyll in their tissues.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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