Amelioration of water deficit by application of potassium and FYM in *Lens culinaris* (L.) Medikus and *Brassica napus* L. under intercropping system

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Abstract
Lens culinaris is an annual edible legume while Brassica napus is a common oil-production crop. One of the most frequent abiotic stresses endangering agricultural food crops is drought. Drought significantly impairs crop plants' physiology and productivity which eventually results in plant death. The present research was done to ameliorate the adverse effects of drought stress on the growth, photosynthetic pigments and water status of Brassica napus L. (GSC-7 variety) and Lens culinaris (L.) Medikus (HM-1 variety) intercropping by combined application of potassium and farm yard manure (FYM). The treatments were given as control (2 irrigations), early irrigation (irrigation only during vegetative stage), late irrigation (irrigation only during flowering stage) and stress (0 irrigation) along with different concentrations of potassium and FYM i.e. K_0(control) K_1(10kg/acre) and K_2(20kg/acre) K_1M (10kg/acre+10tonne/hectare) and K_2M (20kg/acre+10tonne/hectare.

At the vegetative stage (45DAS), samples were taken and analyzed for different attributes. Drought stress significantly affected the morphology and physiology of both cultivars by reducing the photosynthetic pigments, water status (relative water content [RWC] and membrane stability index [MSI]) in both cultivars compared to the control. But potassium(K) and FYM supplementation improved both cultivars' RWC, MSI, photosynthetic pigments and growth parameters. In addition, after K and FYM application under drought stress, MDA levels and electrolyte leakage were decreased. These results authenticate that potassium in combination with FYM considerably reduces the effect of drought on both cultivars of intercropping by enhancing the plants' growth, photosynthetic activities and water status.

Keywords: Drought stress, Potassium, Photosynthetic pigments, Intercropping, Productivity.

Introduction

Exponential production of agriculture is a necessity to complete the increasing demands of expanding global population. Abiotic stressors which include heat, cold, salinity and drought, are utmost detrimental elements disturbing crop development and yields worldwide[13,14]. Global environmental changes (GEC) affecting agricultural production directly by altering crop yield and quality include drought, flooding, soil acidity, salinization, exceptionally low and high temperatures and other unfavorable environmental circumstances[8]. In all ecological regions of the world, drought, or hydropenia, is the most common abiotic stress that field crops experience. In various countries worldwide, lack of water is the major barrier to agricultural production, influencing crop quality, growth and output[2].

Plants undergo significant changes under drought stress that reduce photosynthesis, canopy size, accelerated leaf senescence, chlorophyll degradation, decreased water nutrient use efficiency and reduced leaf size which are some primary negative impacts that destroy a plant under drought stress[21,22,29]. Additionally, oxidative damage to nucleic acids, enzymes, proteins and cell membranes occurs as a result of reactive oxygen species (ROS) in different plant parts under water deficit stress[9]. Malondialdehyde (MDA) concentration rises as a result of the drought-induced formation of highly reactive oxygen species. MDA concentration is considered a sign of oxidative damage[4].

Normally, plants have a built-in defense mechanism that can only withstand certain levels of stress. The most obvious is the antioxidant defense mechanism which includes non-enzymatic (vitamins, glutathione, polyphenols and ascorbate, polyphenols) and enzymatic components (ascorbate peroxidase, catalase, peroxidase and superoxide dismutase,) which effectively scavenge the drought-caused ROS in plant cells[27,30]. Under extreme and protracted stress conditions, the natural defense system is compromised which results in physiological anomalies such as decreased leaf area, plant height, growth rate, photosynthesis, water potential and stomatal conductance.

The prolonged severe stress situations activate the defense system of plants naturally and leads to some disturbances in physiological attributes as reduction in stomatal conductance, growth rate, water potential, reduced photosynthesis and plant height[31]. Potassium (K) is crucial to crop growth and productivity[5,26]. It is a crucial nutrient for both the transfer of assimilates and photosynthesis[33]. By facilitating the transfer of assimilates and preserving osmotic charge, potassium influences the osmotic adjustment of the plant boosting drought tolerance[11,24,25].

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Farmyard manure (FYM) is a significant organic resource for agricultural productivity in semi-arid locations with livestock-based farming systems. Organic manure application improves the biological, physical and chemical condition of the soils and supplies all the nutrients (macro and micronutrients) required by the plant as this manure is produced from cow dung, cow urine, dairy wastes and waste straw[19]. The synergistic effects of both fertilizers and organic manures increase the amount of total available nitrogen in the field soils[17]. The application of organic amendments along with inorganic fertilizer enhanced the soil N, P and K contents[15].

The present study aims to determine different irrigation regimes' effects and management strategies using potassium and farm yard manure (FYM) application in lentil and oilseed rape varieties HM 1 and GSC 07 respectively under the intercropping system.

Material and Methods

Experimental site and soil analysis: The field study was carried out during the rabi season of 2021-2022 and 2022-2023 in the nursery of Kurukshetra University, Kurukshetra, India (29˚95'N; 76˚82'E). Soil samples of the experimental area were air-dried, passed through a sieve and examined. The physicochemical characteristics of the experimental soil consisted of organic carbon (0.95%), P (12.8 ppm) and K (206 ppm). The pH of the soil was found to be 7.4 and EC was 1.55(dS/m).

Plant material and stress treatments: The seeds of Gobhi sarson variety GSC 7 were procured from the oil seed section of Punjab Agricultural University, Punjab and the seeds of lentil variety HM 1 were procured from the pulses section of CCSHAU (Chaudhary Charan Singh Hisar Agricultural University), Hisar, Haryana. The experimental study was split into 10 treatments with three replications of each treatment using a randomized complete block design with two factors. One factor was different irrigation intervals alongside control and another factor consisted of different concentrations of potassium and constant concentration of FYM (Table 1). The crops were grown as an intercrop with 1:1 row intercropping in blocks of 2sq.m each.

Determination of Growth parameters: After 45 days of sowing, both the cultivar's samples were taken from each treatment's replicate, the shoot length was measured with a measuring scale and their averages were used for further analysis. The shoot length was defined as the length of the plant from the soil surface and expressed in cm. The leaf area of plants (sq.cm.) was determined with the help of leaf area meter (Systronics 211, Ahmadabad, India).

Physiological parameters: Leaf relative water content served as a measure of the leaves' water status (RWC) done by Weatherly[35]. To obtain the fresh weight, the leaves were weighed right away (FW). The leaves were then immersed for 4 hours in distilled water to determine the turgid weight (TW).

After that, leaves were oven dried for 48 hours at 72 degrees to determine the dry weight (DW). The RWC was measured by formula:

RWC = [(FW DW)/ (TW DW)] 100.

Table 1
Experimental details of different treatments with different irrigation regimes with K and FYM application.

K+ FYM conc. / No. of irrigations	2 (Control)	1 (Early Irrigation)	1 (Late irrigation)	0 (Stress)
0 + 0	C+KO	EI + KO	LI + KO	S + KO
10kg/acre + 0	C + K1	EI + K1	LI + K1	S + K1
20kg/acre + 0	C + K2	EI + K2	L1 + K2	S + K2
10kg/acre + 10tonnes /hectare	C + K1M	EI + K1M	LI + K1M	S + K1M
20kg/acre + 10tonnes /hectare	C + K2M	EI + K2M	LI + K2M	S + K2M

Electrolyte leakage was measured using an electrical conductivity meter as demonstrated by Lutts et al.[24] The initial step was to extract the fresh leachates and soak them in deionized water before drying them with filter paper. 1g of freshly harvested leaves was chopped. Cut the tissues into tiny pieces (approximately 1 cm^2), drain all the electrolytes and then soak them in 20 ml of deionized water. To thoroughly kill the tissues and remove all electrolytes, the bathing solution was subsequently autoclaved for 20 minutes. The electrical conductivity (EC1) was then calculated. After being cooled, the samples' electrical conductivity (EC2) was then assessed.

The membrane stability index (MSI) was calculated based on the electrolytic leakage of leaves by Deshmukh et al[10]. The electrical conductivities of leachates present in DDW (double-distilled water) at 40 °C and 100 °C were examined to determine the amount of electrolyte loss. Cut to the same size, two identical (0.1-gram) discs were placed in two distinct test tubes with double-distilled water (10ml). A conductivity meter was used to measure the two discs' various electric conductivities C1 and C2 after they were each stored at different temperatures for 30 minutes—one at 40 °C and the other at 100°C (Digital Conductivity Meter, HP G-3001). The following formula was used to calculate it:

$$\text{MSI (\%)} = [1-(C1/C2)] \times 100$$

Biochemical parameters

Total chlorophylls estimation: A leaf sample (0.1g) was extracted with 80% acetone and then centrifuged at 5000 rpm for 20 minutes. Absorbance was recorded spectrophotometrically at 645,663 and 470nm by the Arnon[6] method. For carotenoids, the absorbance was recorded at both 480nm and 510nm.

The equations below determine chlorophyll concentration:

Total chlorophyll = Chlorophyll a + Chlorophyll b

$$\text{Chlorophyll a (mg/g FW)} = \frac{12.3(A_{663})-0.86\,(A_{645}) \times V}{\alpha \times 1000 \times W}$$

$$\text{Chlorophyll b (mg/g FW)} = \frac{19.3(A_{645})-3.6(A_{663}) \times V}{\alpha \times 1000 \times W}$$

$$\text{Carotenoids content} = 7.6(A480)-1.49(A510) \times (V/d\times 1000\times W)$$

MDA content: A modified thiobarbituric acid (TBA) method was used to measure the malondialdehyde (MDA) level by Hodges et al[16]. Fresh leaves weighing 0.2 g were homogenized in trichloroacetic acid (5ml) at a concentration of 0.1% (w/v) for 10 minutes. 1-mL aliquot of the supernatant was mixed with trichloroacetic acid (20%)/TBA (0.5%) solution in four milliliters. This mixture was heated to 100 °C for 15 minutes, allowed to cool on ice for 10 minutes and then centrifuged at 10,000 g. At 450, 532 and 600 nm, the supernatant's absorbance was measured:

$$\text{MDA content} = \frac{\text{Absorbance} \times \text{Total volume (ml)} \times 1000}{\text{Extinction coefficient} \times \text{Volume of the sample (ml)} \times \text{Weight of leaf sample(g)}}$$

where Extinction coefficient = 155 mM^{-1} cm

Statistical analysis: Analysis of Variance (ANOVA) was used for computing statistical data using software SPSS[16]. The significant differences of each treatment were calculated by Duncan's HSD post hoc test ($p< 0.05$). MS Excel was used to manage the data collected while sampling. Pearson's correlation coefficients analysis was done. To study correlations among different traits, Pearson's correlation coefficients analysis was done using RStudio software.

Results and Discussion

Impact of Potassium and FYM Application on plant growth under water stress: Both crops were significantly affected by irrigation, farm yard manure and potassium levels. Results of interaction of irrigation levels and potassium application on vegetative growth attributes (shoot length and leaf area) are shown in figure 1. The application of potassium fertilizer significantly improved the growth traits of both crops but the maximum increase was with K_1(10kg/acre) as compared to higher concentrations i.e. K_2 (20kg/acre) and the results were best when potassium was applied in combination with FYM. With potassium and FYM application, the shoot length increased by 31.3% in GSC 7 and 28.88% in lentils under stress($S+K_1M$) as compared to stress control ($S+K_0$). The leaf area was lowest in the treatments: S + K_0, LI + K_0 and C + K_0. The leaf area was increased by up to 80.86% and 18.8% in GSC 7 and HM 1 of oilseed rape and lentil respectively under stress conditions with treatment(S+K1).

The supplementation of potassium has a great effect on different physiological processes involved in growth and development of plant and varying amount of organic manure enhances water retention capacities of soil. The same results were observed in Brassica juncea (L.) Czern cultivars where plant growth attributes showed an increase with an increase in potassium sulfate concentration[28]. Hussain et al[18] revealed that varying concentrations of potassium (0, 30, 60, 90 and 120 kg sulphate of potash (SOP) ha-1) had a profound effect on plant height in case of mungbean cropping system.

Impact of Potassium and FYM Application on plant water status under water stress: In sugar beets under drought stress, Aksu and Altay[3] showed that potassium treatment increased the RWC content and decreased the MDA concentration, reducing damage to the membrane. Applying potassium reduced the drought susceptibility in hybrid maize by increasing water productivity, especially when there was water stress[32]. These results were in line with our study. The RWC and MSI were drastically reduced with an increase in water stress (Figure 2) whereas there was an increase in electrolyte leakage and MDA content (Figure 3). Compared to full control ($C+K_0$), the RWC of leaves declined up to 5.58% in the HM 1 variety of lentils and 13.9% in the GSC 7 variety of oilseed rape at complete stress ($S+K_0$).

Additionally, potassium(K) application improved the RWC of leaves in interaction with Farm Yard Manure (FYM) with the maximum at K_1M application by up to 3.35% in HM 1 and 21.9% in GSC 7 under stress ($S+K_1M$). The maximum MSI was observed in treatment $C+K_1M$ (84.45%) in GSC 07 and in treatment $EI+K_1M$ (94.63%) in HM 1 of lentil.

Impact of Potassium and FYM Application on plant electrolyte leakage and lipid peroxidation under water stress: There was an increase in leaves electrolyte leakage by up to 42.55% and 47.77% in GSC 07 of oilseed rape and HM 1 of lentils respectively under stress conditions without fertilizer ($S+K_0$) when compared to control. However, K and FYM had a big role in lowering the harmful impacts of drought by supressing the electrolyte leakage of leaves up to 19.56% and 18.7% in GSC 07 and HM 1 respectively compared to control($S+K_1M$).

By examining the MDA levels in control and drought-stressed plants, lipid peroxidation was quantified. When compared to the control, the MDA content under drought stress was substantially higher. The MDA content in treatment C+K0 was 0.729 ± 0.019 µmol MDA g−1 FW in GSC 07 and 2.476 ± 0.091 µmol MDA g−1 FW in HM1 which exponentially increased to 3.199 ± 0.136 µmol MDA g−1 FW in GSC 07 and 6.45 ± 0.080 µmol MDA g−1 FW in HM 1 by reducing the irrigation intervals ($S+K_0$). However, K and FYM application cause a reduction in these values in both cultivars. Malondialdehyde concentration was increased in barley leaves as a result of salt stress and drought stress and its amount was reduced by KNO_3 applications[12].

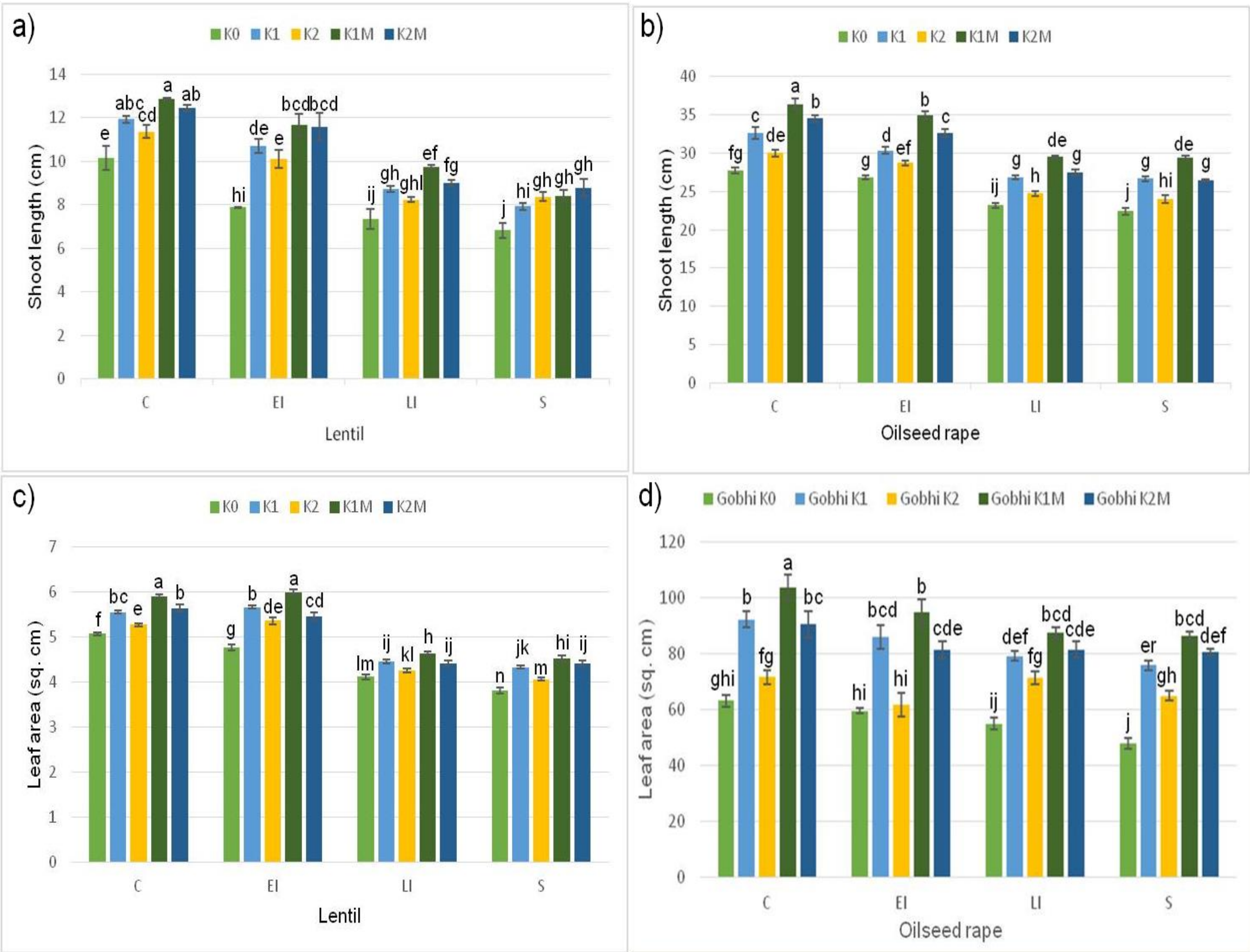


Figure 1: Effect of K and FYM on shoot length of lentil (a) and oilseed rape (b) and on leaf area of lentil (c) and oilseed rape (d) under different irrigation regimes. Small bars denotes the standard error. There was a significant difference between treatments for each bar with a unique alphabet ($p< 0.05$). Different irrigation intervals as C: Control, EI: Early irrigation, LI: Late irrigation, S: Stress with different fertilizer treatments as K_0: 0Kg/acre, K_1: 10kg/acre, K_2: 20kg/acre of K, M: 10 tonnes/hectare FYM

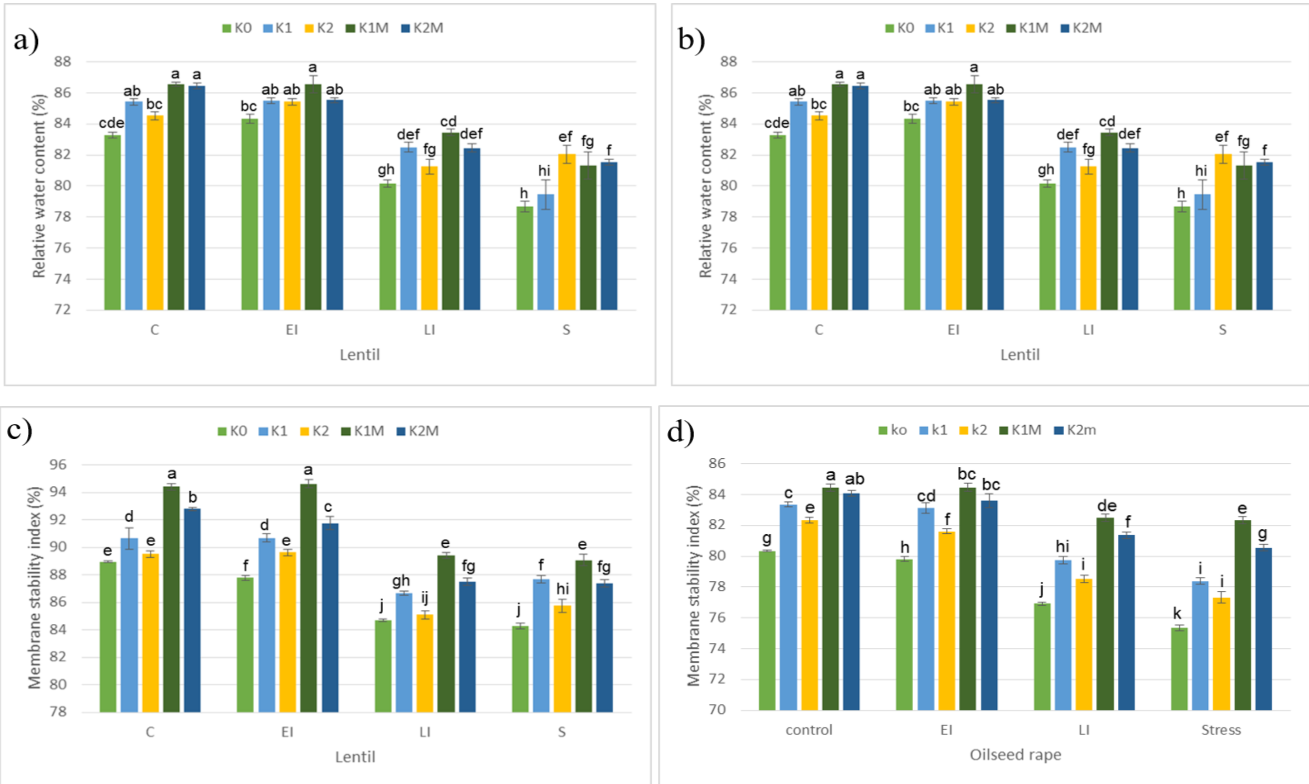


Figure 2: Effect of K and FYM on (a) Relative water content of lentil and (b) oilseed rape and (c) on Membrane stability index of lentil and (d) oilseed rape under different irrigation regimes. Small bars denotes the standard error. There was a significant difference between treatments for each bar with a unique alphabet ($p< 0.05$). Different irrigation intervals as as C: Control, EI: Early irrigation, LI: Late irrigation, S: Stress with different fertilizer treatments as K_0: 0Kg/acre, K_1: 10kg/acre, K_2: 20kg/acre of K, M: 10 tonnes/hectare FYM

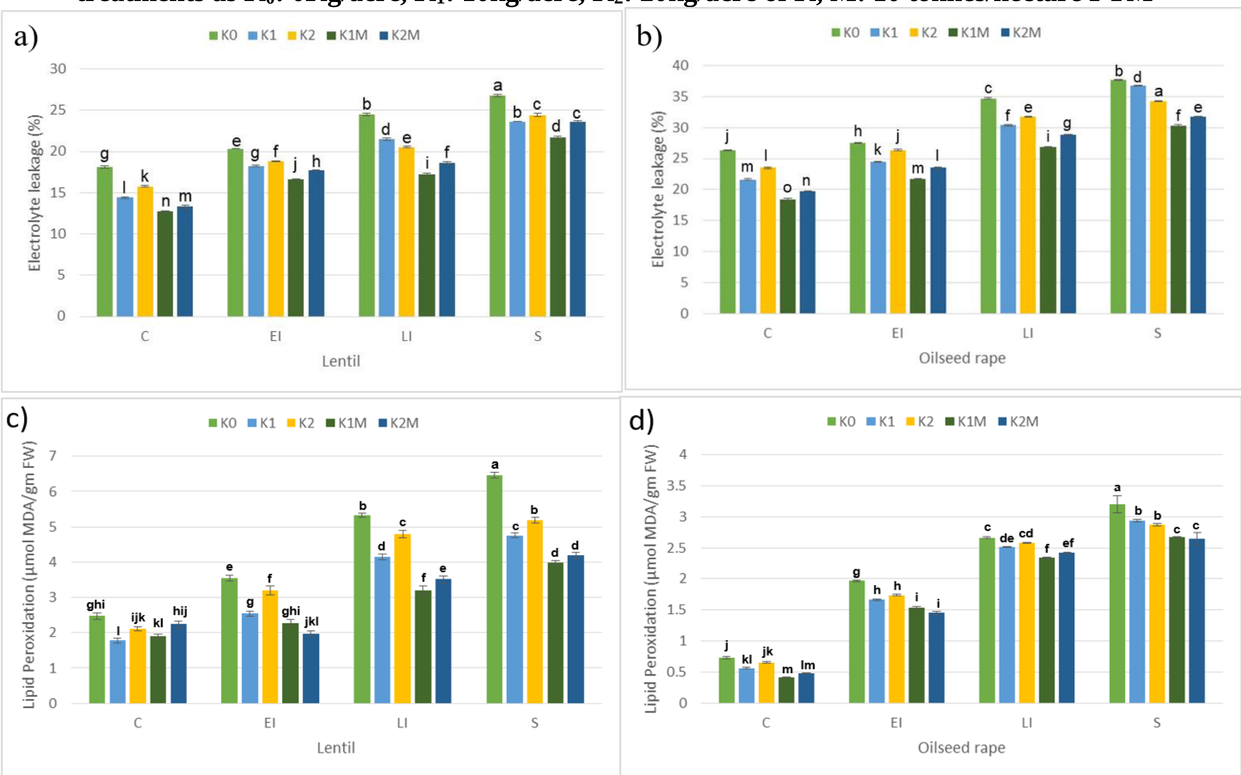


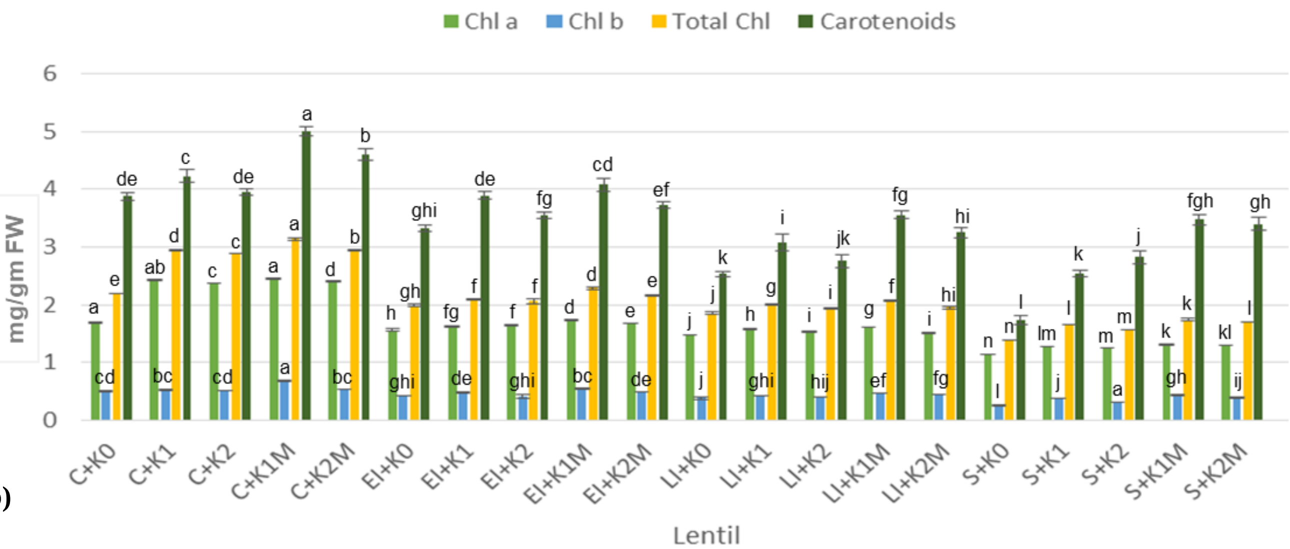
Figure 3: Effect of K and FYM on (a) Electrolyte leakage of lentil and (b) oilseed rape and (c) on Lipid peroxidation of lentil and (d) oilseed rape under different irrigation regimes. Small bars denotes standard error. There was a significant difference between treatments for each bar with a unique alphabet ($p< 0.05$). Different irrigation intervals as C: Control, EI: Early irrigation, LI: Late irrigation, S: Stress with different fertilizer treatments as K_0: 0Kg/acre, K_1: 10kg/acre, K_2: 20kg/acre of K, M: 10 tonnes/hectare FYM

Impact of Potassium and FYM application on plant photosynthetic pigments under water stress: Our present investigation showed a significant reduction in photosynthetic pigments such as total chlorophyll, chl a, chl b and carotenoids under water stress conditions (Figure 4). This was most likely caused by the suppression of the RuBP enzyme and systemic damage to the photosynthetic machinery which ultimately led to a reduction in photosynthetic pigments[1,20].

It was found that with potassium supplementation, there was an increase in photosynthetic pigments with a maximum value obtained with K1M (10 kg/acre) followed by K2M (20 kg/acre). K and FYM supplementation improved chlorophyll a from 0.1677 ± 0.008 mg/g FW (S+K0) to 0.295±0.0056 mg/g FW (S+K1M) in GSC 7 variety of oilseed rape and 1.1403±0.0092 (S+K0) to 1.315 ± 0.00751 mg/g FW (S+K1M) in HM 1 variety of lentil and chlorophyll b from 0.0503 ±0.005(S+K_0) to 0.1063±0.0042 mg/g FW (S+K1M). Likewise, total chlorophyll was also increased with K and FYM supplementation from 0.218±0.0026 (S+K0) to 0.4013±0.00533 mg/g FW (S+K1M) in GSC 7 of oilseed rape and 1.3873±0.0087 to 1.7447±0.0165 mg/g FW (S+K1M) in HM 1 of lentil. Asgharipour and Heidari[7] found that chlorophyll content increased significantly with increasing levels of irrigation and potassium.

Correlation Analysis: Pearson's correlation graph was made by analyzing the relationship among different attributes of growth, physiological, biochemical and photosynthetic pigments of GSC 7 and HM 1 variety of oilseed rape and lentil respectively as in figure 5(a and b). In both the plants under intercropping RWC, total chlorophyll, carotenoid, chlorophyll 'a' and 'b,' leaf area, shoot length and MSI show significant correlation with each other but negatively correlated with electrolyte leakage and MDA content of leaves and vice versa.

a)



b)

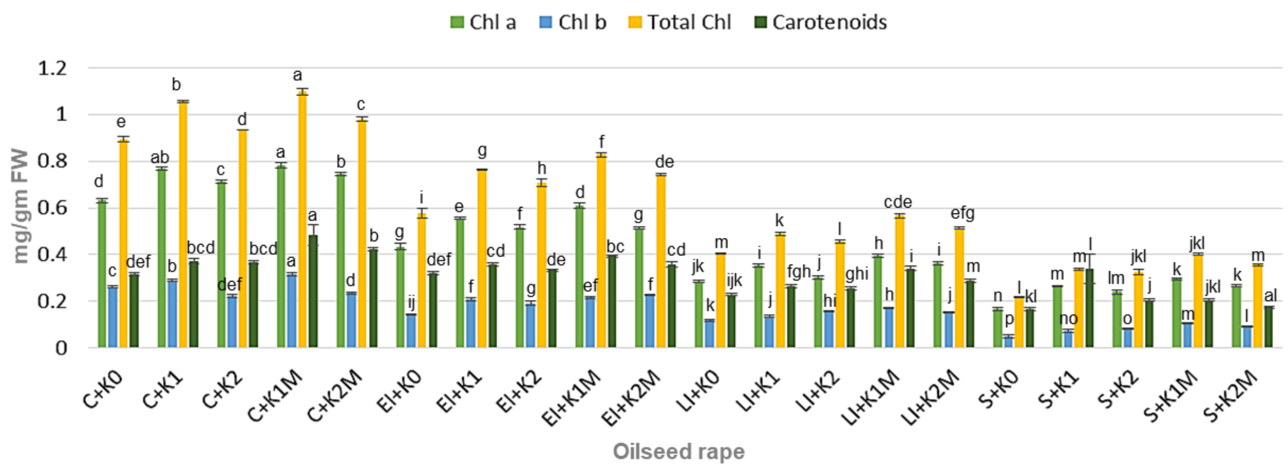


Figure 4: Effect of K and FYM on photosynthetic pigments of lentil (a) and oilseed rape (b) and under different irrigation regimes. Small bars denotes the standard error. There was a significant difference between treatments for each bar with a unique alphabet ($p < 0.05$). Different irrigation intervals as C: Control, EI: Early irrigation, LI: Late irrigation, S: Stress with different fertilizer treatments as K_0: 0Kg/acre, K_1: 10kg/acre, K_2: 20kg/acre of K, M: 10 tonnes/hectare FYM

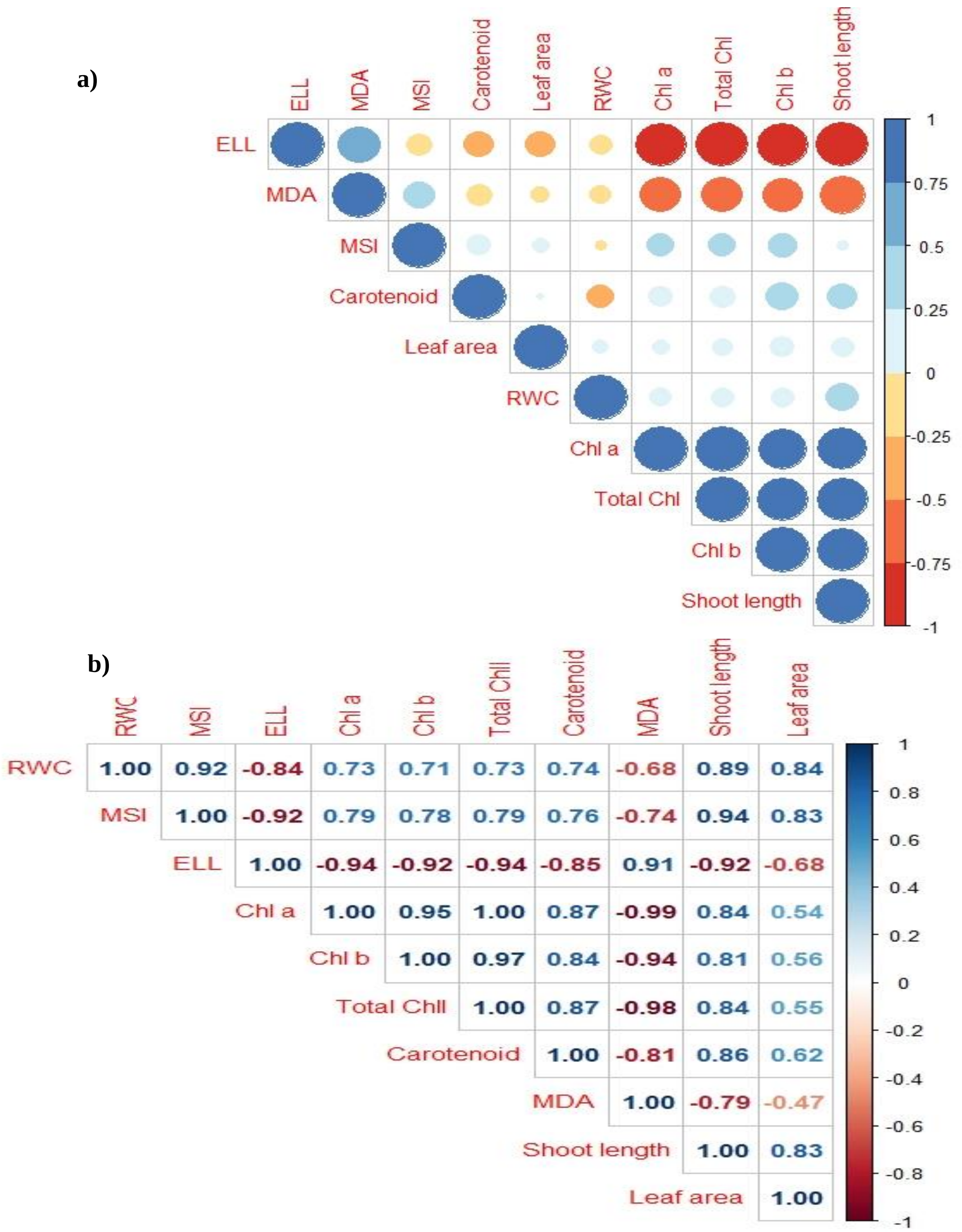


Figure 5: Pearson correlation between different attributes including growth, physiological and, photosynthetic pigments of HM 1 of lentil (a) and GSC 7 of oilseed rape(b) under different water irrigation systems and different potassium and FYM treatment. The blue color intensity showing a positive correlation and the red color intensity showing a negative correlation. RWC: relative water content, ELL: electrolyte leakage of leaves, MSI: membrane stability index. Total chl: total chlorophyll, chl 'a': chlorophyll 'a', chl 'b': chlorophyll b, Carotenoid: Total carotenoid content

Conclusion

Potassium deficiency depressed rapeseed and lentil drought tolerance, leading to a decline in growth, photosynthetic pigments and water status. Potassium separately or in combination with FYM resulted in enhancement in growth, water status and photosynthetic pigments in both cultivars under drought-stressed and control conditions. Results of the present study authenticated that 10 kg K acre^{-1} along with FYM was the best treatment and this trend indicates that organic manure is helpful in drought-affected areas along with a limited supply of inorganic fertilizer reducing the negative effects of chemical fertilizers on the environment.

The results obtained will help increase the water stress tolerance of both cultivars under water deficit conditions in drought-hit areas by supplementation of potassium fertilizer alongside FYM.

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