IMPACT OF POTASSIUM ON PHYSIOLOGICAL CHARACTERISTICS OF LENTIL AND OILSEED RAPE INTERCROPPING UNDER DIFFERENT IRRIGATION REGIMES

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Abstract. Water deficit severely affects crop yield. Potassium is responsible for reducing the hazardous impacts of drought stress on crops. This field study evaluated the impact of potassium application on physiological and biochemical attributes of *Lens culinaris* (L.) Medikus (lentil) and *Brassica napus* L. (oilseed rape) cultivars under the intercropping system. HM-1 variety of lentil and GSC 07 variety of oilseed rape variety were grown with different concentrations of potassium (K_0 - 0 kg × acre^{-1}, K_1 – 10 kg × acre^{-1} and K_2 – 20 kg × acre^{-1}) subjected to different irrigation regimes (control, early irrigation, late irrigation and stress). The results demonstrated that water deficit greatly reduced photosynthetic pigments and physiological attributes (RWC and MSI) over control. However, antioxidant enzyme activity and electrolyte leakage were enhanced in water deficit. The field study indicates that the application of potassium was effective in alleviating the drastic effects of water deficit on both the cultivars.

Keywords: potassium; photosynthetic pigments; water deficit; antioxidant enzymes.

INTRODUCTION

Globally sustainable agricultural output is being seriously threatened by the current rapid climate change,which has also made it difficult for many nations to experience economic growth [4, 50, 53]. Abiotic stresses, such as extreme temperatures, drought, salinity, heavy metals etc are the main obstacles in the growth and development of plants [12, 19]. Reduced stomatal opening, impaired CO_2 fixation, accelerated O_2 photoreduction in the chloroplast, and increased photorespiration are all effects of water deficiency stress that eventually cause reactive oxygen species (ROS) buildup and ultimately oxidative stress in plants [23]. In order to maintain optimal development under water deficiency conditions, plants have developed sophisticated systems for adaptation to drought stress [16]. To mitigate the adverse effects of oxidative stress, plants possess extraordinarily effective antioxidant machinery consisting of enzymatic and non-enzymatic constituents [18, 43].

One of the most important problems in the development of plant products is the management of plant nutrition under stressful situations [25]. The three main essential nutrients for plant growth are potassium (K), nitrogen (N), and phosphorus (P) where K plays a major role in strengthen plants' abilities [41]. However, under drought stress, the availability of different nutrients in the soil changes significantly, upsetting the balance of nutrients that the plant absorbs and making necessary elements less available to the plant [25].

Potassium is a crucial macronutrient having roles in osmoregulation, membrane potential regulation, and contransport of sugars, with a wide range of impacts on the control of plant growth, metabolism, reproduction, and stress tolerance under difficult settings [38]. It is the fourth most prevalent metal in the earth's crust, although only a small percentage (1-4%) of it is accessible for plant uptake [17]. Through osmotic adjustment related to membrane potential, enzyme activation, water absorption, and cell turgor maintenance, K application plays a crucial role in the development of resistance mechanisms in plants by enzyme activation and maintenance of cell turgor [2, 36]. Lack of K causes damage to membranes and chlorophyll in plants, which in turn affects photosynthetic CO_2 fixation, photoassimilates transport, and consumption [42].

Lens culinaris (L.) Medikus is the second most important legume crop in India after chickpea belonging to family Fabaceae grown during the winter. As a legume, it fixes atmospheric nitrogen through *Rhizobium* bacteria-produced root nodules, which lessens the need for applying nitrogenous fertilizers [21]. Due to its most affordable and concentrated type of nutritional protein, it is considered a "poor man's meat". It contains 23.25 percent protein, 59% carbohydrates, 1.8% oil, 0.2% ash and has traces of iron, calcium, phosphorus, and magnesium. The flour can be combined with cereal flour and used to make cakes, breads, and various baby feeds. The straw makes excellent animal feed, the seed of the plant is also a rich source of vitamins and minerals for human use [31, 33, 44].

With 435 genera and 3675 species worldwide, the Brassicaceae family is one of the largest [35]. An important source of edible oil, *Brassica napus* L. (Oilseed rape) is a model plant for winter cover crops, and its population is in demand worldwide. It is also a promising crop for biodiesel production [55]. In rapeseeds, more than 25% protein, 61% oleic acid and 8.8% linoleic acid are found in comparison to other oil seeds. The canola oil is considered a very healthy containing 27% multi-unsaturated acids, 66% mono-unsaturated acids, and 7% saturated fatty acids [26].

Intercropping aims to harness the principles of competition, facilitation, and complementarity, which categorizes many types of interspecies interactions that takes place in multi-species stands, to increase crop growth and system productivity [6, 29].

The present investigation was therefore, undertaken to evaluate the impact of potassium application on physiological and biochemical attributes of HM-1 variety of *L. culinaris* and GSC 07 variety of *B. napus* cultivars under the intercropping system.

MATERIALS AND METHODS

Soil characteristics: The study was carried out to determine the role of K [0, 10 and 20 kg × acre^{-1} as Muriate of Potash (KCl)] in improving drought tolerance of HM-1 variety of *L. culinaris* and GSC 07 variety of *B. napus* cultivars under different irrigation regimes and sampling was done 65 days after sowing (DAS). The field experiment was done in plant nursery of horticulture department, Kurukshetra University, Kurukshetra (29˚95'N; 76˚82'E) from October to March, 2021-2022.The soil tested for the field experiment was clay loamy in texture and characteristics were as follows: P (12.8 ppm), K (206 ppm) carbon (0.95%), alkaline (pH- 7.4) and EC was 1.55(dSm^{-1}).

Experimental setup: The seeds of oilseed rape variety GSC 07 were bought from the oil seed section of Punjab Agricultural University, India, and lentil variety HM-1 from the pulses section of Chaudhary Charan Singh Hisar Agricultural University, India. The experiment was divided into ten treatments having three replications of each using a randomized complete block design. Four different irrigation levels [control (both irrigations), early irrigation (irrigation only during vegetative stage), late irrigation (irrigation only during flowering stage), and stress (no irrigation) were taken with three different concentrations of Potassium (00, 10 and 20 kg × acre^{-1} as Muriate of Potash) as given in Table 1. The crops were grown as an intercrop with 1:1 row intercropping in blocks of 2 m^2 each having interspacing of 25 × 15 cm.

Physiological characteristics: In order to determine the water state of the leaves, leaf-relative water content (RWC) was calculated using the method given by Weatherly [50]. The leaves were weighed immediately to determine the fresh weight (FW). The turgid weight (TW) of the leaves was then calculated after being submerged in distilled water for 4 hours. To calculate the dry weight (DW), leaves were oven dried for 48 hours at 72°C. The RWC was calculated using the equation, RWC = [(FW DW)\(TW DW)] 100.

Electrolyte leakage was measured using an electrical conductivity meter following the method of Lutts *et al*. [31]. The fresh leachates were first extracted, soaked in deionized water, and then dried with filter paper. Freshly picked leaves weighing 1g were cut. The tissues should be divided into little (1 cm^2) pieces. After draining the electrolytes, the tissues were then soaked in 20 mL of deionized water. The bath solution was then autoclaved at 120°C for 20 minutes to completely destroy the tissues and eliminate any electrolytes. Then, the electrical conductivity (EC1) was determined. The samples' electrical conductivity (EC2) was then measured after cooling to 25°C.

The membrane stability index (MSI) was calculated using the electrolytic leakage of leaves [11]. To calculate the rate of electrolyte loss, the electrical conductivities of leachates present in DDW (double-distilled water) at 40 °C and 100 °C were analysed. Two identical discs (0.1 g) were cut to the same size and inserted in two separate test tubes (10 mL) with double-distilled water. After being held at two distinct temperatures for 30 minutes, one at 40°C and the other at 100°C, the two discs' differing electric conductivities, C1 and C2, were measured using a conductivity metre (Digital Conductivity Metre, HP G-3001). It was computed using the formula below:

MSI (%) = [1- (C1/C2)] x 100

Biochemical characteristics: The Arnon method [5] was used for the estimation of chlorophyll a (mg/g FW), chlorophyll b (mg/g FW) and carotenoids content. Total chlorophyll equals the sum of chlorophylls a and b.

Preparation of enzme extract: Using a mortar and pestle, a 100 mg sample of tissue was taken and homogenized in 2 mL of 0.1 M EDTA phosphate buffer (pH 7.8) before being spun at 1500 rpm. The reaction mixture was next tested for the activity of several antioxidant enzymes.

Superoxide dismutase (SOD) was measured by the following method of Giannopolitis and Ries [14]. To make the final reaction mixture of 3 mL with DDW, add 0.1 mL of the enzyme mixture, 0.5 mL of Na_2CO_3 (300 Mm, pH 10.2), 0.5 mL of 378 μM p-nitrobluetetrazolium chloride (NBT), 0.5 mL of L-

Table 1. Treatments in the experiment

Treatment	Code	Details
T1	C	Control (2 irrigations: first at vegetative stage; second at flowering stage)
T2	EI+K_0	Early irrigation (irrigation at vegetative stage only) +K_0
T3	EI+K_1	Early irrigation (irrigation at vegetative stage only) +K_1
T4	EI+K_2	Early irrigation (irrigation at vegetative stage only) +K_2
T5	LI+K_0	Late irrigation (irrigation at flowering stage only) + K_0
T6	LI+K_1	Late irrigation (irrigation at flowering stage only) + K_1
T7	LI+K_2	Late irrigation (irrigation at flowering stage only) + K_2
T8	S+K_0	Stress (no irrigation) +K_0
T9	S+K_1	Stress (no irrigation) +K_1
T10	S+K_2	Stress (no irrigation) +K_2

methionine (78 mM), and 0.5 mL of riboflavin (7.8 µM). The absorbance was determined at 560 nm, and one SOD unit is equal to the amount of enzyme that prevents NBT from being reduced by 50% .

Hydrogen peroxide (H_2O_2) breakdown caused a decrease in absorbance at 240 nm, which was used to measure the catalase (CAT) activity. A 1.5 mL of phosphate buffer solution (100 mM, pH 7.0), 1.2 mL of H_2O_2 (150 mM), and 0.3 mL of enzyme extract made up the reaction mixture [3].

By mixing 100 µL of the enzyme extract with 1.8 mL of potassium phosphate buffer (50 mM, pH 7.6), 300 mL of EDTA (3 mM), 0.1 mM NADPH, and 1 mM GSSG, glutathione reductase (GR) activity was measured. After increasing the total volume to 3 mL, the absorbance was measured at 340 nm [9].

Statistical analysis: Statistical analysis was performed with analysis of variance (ANOVA) by using the software Statistical Package for Social Sciences (ver. 16, Chicago, IL). The significant differences of each treatment were calculated by Duncan's post hoc test ($p < 0.05$). MS Excel was used to manage the data collected while sampling.

RESULTS

In this research, K as fertilizer significantly improved the physiological and biochemical traits of both the crops under water deficit conditions (Fig. 1). There was a decline in RWC with a decrease in irrigation intervals and it was lowest (54.37 and 66.75%) at treatment S+ K_0 for oilseed rape and lentil respectively. The maximum RWC was observed in treatment C (66.32% and 78.38%) followed by EI+K_1 (65.74% and 77.91%) in oilseed rape and lentil respectively.

The MSI declined from 64.01% (C) to 51.02% under stress in oilseed rape and similar trend was observed in lentil. After the application of potassium supplementation, an increase in MSI by upto 5.71% (S+K_1) was observed in oilseed rape and 4.7% (S+K_1) in lentil over control. While, the electrolyte leakage of leaves was increased by upto 32.98% in oilseed rape and 43.98% in lentil under stress conditions. However, K supplementation suppressed the electrolyte leakage of the leaves by upto 8% in oilseed rape and 14.86% in lentil under stress.

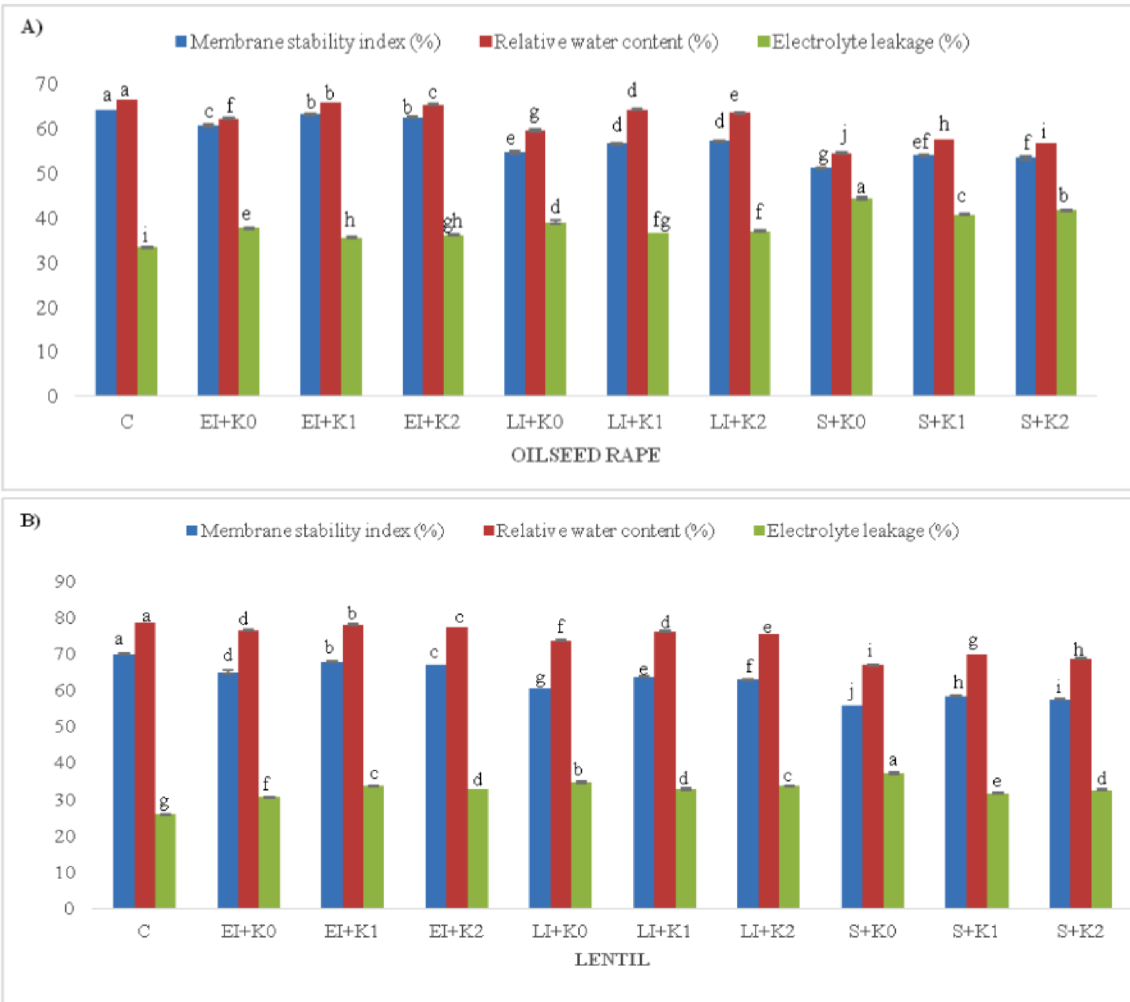

Figure 1. Effect of Potassium on Membrane Stability Index (MSI), Relative Water Content (RWC) and Electrolyte Leakage (EL) on: (a) Oilseed rape and (b) Lentil plants under different irrigation regimes. (Small bars in the figure show the standard error). There was a significant difference between treatments for each bar with a unique alphabet ($p < 0.05$). C - control, EI -early irrigation, LI - late irrigation, S - stress, K_0 – 0 kg × acre^{-1}, K_1 – 10 kg × acre^{-1}, K_2 – 20 kg × acre^{-1} of K.

The results in Fig. 2 revealed the interaction of water deficit conditions and antioxidant enzymes. The antioxidant enzymes levels increased with increasing stress conditions and was maximum in case of CAT but SOD and GR activity were lower at stress over LI. K and FYM application increased all the antioxidant enzymes activities at a par value. The SOD activity was highest at LI+K_1 (31.093±0.150) and (34.593±0.231) in oilseed rape and lentil respectively over control (9.99±0.223 and 12.433±0.124) in oilseed rape and lentil respectively. When compared to control, the GR activity showed an increase from 1.831±0.004 to 3.807±0.009 in oilseed rape and 3.537±0.031 to 4.714±0.042 in lentil under late irrigation conditions (LI+K). Surprisingly, the catalase activities were highest in treatment S+K_1 followed by S+K_2 as compared to control (C). Application of potassium increased the catalase activity by upto 11.42% in oilseed rape and 9.48% in lentil under stress conditions (S+K_1).

Water deficit conditions leads to destruction of chlorophyll pigments leading to a decrease in photosynthetic pigments (Table 2 & 3). It was found that with potassium supplementation, there was an increase in photosynthetic pigments with a maximum value obtained with K_1 followed by K_2. K supplementation improved chlorophyll a from 0.464±0.0221 mg × g^{-1} FW (S+K_0) to 0.6387±0.0219 mg × g^{-1} FW (S+K_1) in oilseed rape and 1.318±0.0037 (S+K_0) to 1.4233±0.005 mg × g^{-1} FW (S+K_1) lentil and chlorophyll b from 0.1363±0.0103 (S+K_0) to 0.176±0.0049 mg × g^{-1} FW (S+K_1) in oilseed rape and 0.3203±0.0052 to 0.4233±0.0057 mg × g^{-1} FW (S+K_1) in lentil. Likewise, there was an increase in total chlorophyll content with K supplementation from 0.6003±0.0241 (S+K_0) to 0.8147±0.0222 mg × g^{-1} FW (S+K_1) in oilseed rape and 1.6383±1.638 to 1.846±0.0078 mg × g^{-1} FW (S+K_1) in lentil. The carotenoids contents was also reduced by upto 61.14% in oilseed rape and 50.86% in lentil at stressed conditions. Potassium application increased the carotenoids content by upto 25.44% in oilseed rape and 27.32% in lentil (S+K_1).

DISCUSSION

Moderate water deficit causes significant alterations in the growth and productivity of significant crop species, while severe deficit may lead to plant death [13]. The stable photosynthetic performance is considered to be vital for healthy plant growth and development by facilitating the conversion of light energy into chemical energy [27]. Drought inhibits the photosynthetic machinery of plants, causes changes in chlorophyll contents and components and damage to the photosynthetic apparatus [8, 37]. It is predictably connected with increased oxidative stress due to enhanced accumulation of ROS, mainly O_2^- and H_2O_2 in chloroplasts, mitochondria, and peroxisomes [10]. Tourian *et al.* [45] also reported a reduction in total chlorophyll content in *Agropyron repens* leaves under water-stress conditions. Short-term water deficit condition causes a negative impact on light harvesting protein complexes, photosystem I (PSI) and photosystem II (PSII) of thylakoid membranes, electron transport chain (ETC), and chlorophyll (Chl)

Table 2. Effects of different irrigation schedules and potassium fertilizer on total chlorophylls and carotenoids on oilseed rape under intercropping system.

Treatments	Parameters (mg × g^{-1} FW)			
	Chlorophyll- a	Chlorophyll- b	Total Chlorophyll	Carotenoids
C	1.280±0.019^a	0.371±0.009^a	1.653±0.028^a	0.435±0.009^a
EI+K_0	0.907±0.008^d	0.239±0.002^d	1.147±0.008^d	0.366±0.003^d
EI+K_1	1.188±0.019^b	0.296±0.004^b	1.484±0.0150^b	0.411±0.005^b
EI+K_2	0.991±0.016^c	0.2673±0.005^c	1.259±0.021^c	0.386±0.003^c
LI+K_0	0.728±0.028^f	0.202±0.007^e	0.930±0.032^e	0.258±0.004^g
LI+K_1	0.940±0.012^{cd}	0.278±0.008^{bc}	1.218±0.015^c	0.311±0.005^e
LI+K_2	0.85±0.019^e	0.243±0.004^d	1.093±0.015^d	0.285±0.004^f
S+K_0	0.464±0.022^i	0.136±0.010^g	0.6003±0.024^h	0.169±0.004^i
S+K_1	0.638±0.021^g	0.176±0.004^f	0.814±0.022^f	0.212±0.004^h
S+K_2	0.565±0.012^h	0.140±0.009^g	0.706±0.022^g	0.184±0.004^i

Values with the same letter in the same column don't differ significantly (P < 0.05)
C - control, EI - early irrigation, LI - late irrigation, S - stress, K_0 – 0 kg × $acre^{-1}$, K_1 – 10 kg × $acre^{-1}$, K_2 – 20 kg × $acre^{-1}$ of K.

Table 3. Effects of different irrigation schedules and potassium fertilizer on total chlorophylls and carotenoids on lentil under intercropping system.

Treatments	Parameters (mg × g^{-1} FW)			
	Chlorophyll- a	Chlorophyll- b	Total Chlorophyll	Carotenoids
C	1.951±0.016^a	0.718±0.003^a	2.669±0.019^a	4.091±0.035^b
EI+K_0	1.629±0.012^{de}	0.528±0.003^d	2.157±0.009^d	3.652±0.020^d
EI+K_1	1.724±0.012^b	0.587±0.005^b	2.312±0.011^b	4.209±0.045^a
EI+K_2	1.686±0.006^c	0.564±0.004^c	2.250±0.007^c	3.997±0.018^c
LI+K_0	1.564±0.003^f	0.432±0.003^g	1.996±0.003^g	3.155±0.013^e
LI+K_1	1.618±0.003^{de}	0.5023±0.005^e	2.120±0.008^e	3.956±0.012^c
LI+K_2	1.594±0.006^e	0.4753±0.006^f	2.069±0.01^f	3.584±0.013^d
S+K_0	1.318±0.003^i	0.3203±0.005^i	1.638±0.008^j	2.073±0.025^h
S+K_1	1.423±0.005^g	0.423±0.005^g	1.846±h0.007^h	2.640±0.019^f
S+K_2	1.385±0.005^h	0.391±0.008^h	1.776±0.009^i	2.541±0.022^g

Values with the same letter in the same column don't differ significantly (P < 0.05)
C - control, EI - early irrigation, LI - late irrigation, S - stress, K_0 – 0 kg × $acre^{-1}$, K_1 – 10 kg × $acre^{-1}$, K_2 – 20 kg × $acre^{-1}$ of K.

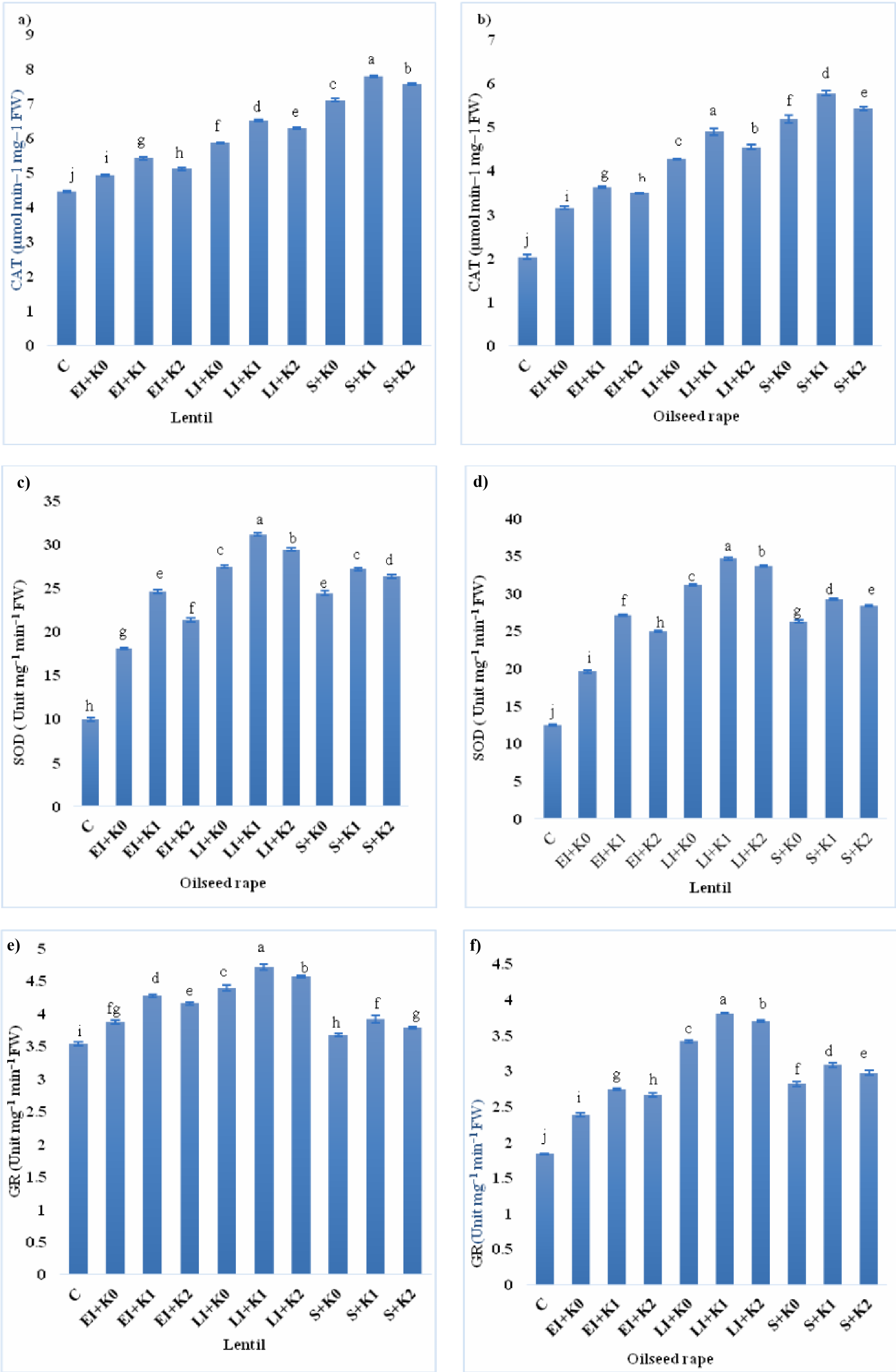

Figure 2. Effect of Potassium on Catalase (CAT) activity of: (a) lentil; (b) CAT activity of oilseed rape; (c) superoxide dismutase (SOD) activity of lentil; (d) SOD activity of oilseed rape; (e) glutathione reductase (GR) activity of lentil; (f) GR activity of oilseed rape under different irrigation (Small bars in the figure show the standard error). There was a significant difference between treatments for each bar with a unique alphabet ($p < 0.05$). C - control, EI -early irrigation, LI - late irrigation S - stress, K_0 – 0 kg × $acre^{-1}$, K_1 – 10 kg × $acre^{-1}$, K_2 – 20 kg × $acre^{-1}$ of K.

biosynthesis. Kalaji *et al.* [27] also observed an increase in chlorophyll contents of well-watered plots in Syrian barley landraces. It may be due to role of K^+ in regulating the manufacture of rubisco, stability of chloroplasts, and the fluxes of nutrients, water, and gases [46]. The use of K improves tolerance to osmotic stress and also increases the activity of many antioxidant enzymes [24, 34]. A low doses of potassium reduced photosynthetic rate, while potassium sulfate application increased the photosynthetic rate by 28% as compared to control in maize [49].

Under stressful circumstances, cell membranes are subject to change, which is typically accompanied by an increase in the cell's permeability leading to an increase in the leakage of ions [22]. An adequate supply of K is necessary to improve drought resistant through root elongation and maintaining cell membrane stability [47]. In *Spinacia oleracea,* potassium and salicylic acid increased root length and decreased electrolyte leakage under water stress [15]. Wasaya *et al.* [48], also reduced the detrimental impacts of drought through K application in *Z. mays'* leaves and reported an improvement in plant's growth characteristics and relative water content. There was a reduction in relative water contents and osmotic potential with low K input and also improved the water status in both cultivars [40]. A mutual interconnection at the transcription level between aquaporins and K^+ transporters has been observed in maize [52], and the transcripts encoding aquaporins were strongly affected by K^+ starvation, even without water stress [30].

Plants have an effective adaptive strategy that makes use of a variety of organic solutes to mitigate environmental challenges [51]. Different plant species, activate both non-enzymatic (such as ascorbate and glutathione) and enzymatic (such as dehydroascorbate reductase, catalase, ascorbate peroxidase, superoxide dismutase, monodehydroascorbate reductase, and glutathione reductase) antioxidant systems in response to water stress [28]. An increase in the activities of different antioxidant enzymes mitigate the detrimental effects of salt stress [54], while the application of exogenous K enhanced the reactive oxygen species scavenging by antioxidants (such as SOD, CAT, and GPX) [39]. An enhanced POD activity catalyze the conversion of H_2O_2 to water and O_2. The overexpression of SOD, if accompanied by enhanced H_2O_2 scavenging mechanisms has been considered as a better protection mechanism against oxidative damage under water stress [1, 10]. The presence of APX and CAT in rapeseed plants also reduced the negative effects of oxidative stress and promoting plant growth significantly [32]. K^+ limits the production of ROS by blocking NADPH oxidase [7]. A direct correlation between oxidative stress and potassium shortage, exhibiting a significant shift in the concentrations of anti-ROS enzymes and an increase in ROS species has been observed in tomato plants during K^+ deficiency [20].

In conclusion, a substantial reduction in various plant pigments (like chl a, b, and carotenoids) at different water status (RWC and MSI) was observed with an increase in electrolyte leakage and antioxydant enzyme activities (SOD,GR and CAT) under water deficit conditions over control. Our research also revealed that K fertilisation improved photosynthetic pigments and water relations by reducing the electrolyte leakage from the membrane.

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