Agricultural Water Management 164 (2016) 83–90

Contents lists available at ScienceDirect

Agricultural Water Management

journal homepage: www.elsevier.com/locate/agwat

Effects of potassium rates and types on growth, leaf gas exchange and biochemical changes in rice (*Oryza sativa*) planted under cyclic water stress

Nurul Amalina Mohd Zain[a,b], Mohd Razi Ismail[b,*]

[a] Institute of Biological Science, Faculty of Science, University Malaya, Kuala Lumpur, Malaysia
[b] Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan Malaysia

ARTICLE INFO

Article history:
Received 13 March 2015
Received in revised form
14 September 2015
Accepted 21 September 2015
Available online 9 October 2015

Keywords:
Cyclic water stress
Potassium
Growth and yield
Leaf gas exchange responses
Biochemical changes

ABSTRACT

Three levels of potassium rates [80 kg K_2O ha^{-1}, 120 kg K_2O ha^{-1} and 160 kg K_2O ha^{-1}) and two types potassium (KCl and K_2SO_4) on rice under cyclic water stress 15 days and the absolute control (80 kg K_2O ha^{-1} of KCl fertilizer on rice under control flooded) were exposed to rice to investigate the influence of potassium in minimizing cyclic water stress effects in rice. It was found as fertilization rates increased from 80 > 120 > 160 kg K_2O ha^{-1} the production of proline was increased. The increase in proline production was simultaneously enhanced the production of Catalase and Maliondialdehyde. As the potassium rate increased from 80 > 120 > 160 kg K_2O ha^{-1}, the transpiration rate was observed to be increased in both potassium types. The result suggested that high potassium rates would reduce water stress effects by having high transpiration rate. High transpiration rate would increase the nutrient uptake that would repair the damage tissue under water stress thus reduce the oxidative stress of rice under water stress condition. This been showed by high significant positive correlations of transpiration rate with CAT activity (r = 0.871; $p \leq 0.05$), MDA (r = 0.914; $p \leq 0.05$) and Proline (r = 0.842; $p \leq 0.05$). It was found that the increase of K rates by KCl increased NAR higher than the increased K rates in K_2SO_4. This might be due to higher absorption of K element in rice by KCl compared to K_2SO_4. The study has showed that application of potassium either KCl or K_2SO_4 would minimize the effects on rice growth and physiology under cyclic water stress. The current study also suggested that plant tolerate to cyclic water stress by increased the production of proline, MDA and decrease of Catalase activity to protect the plant from damage from water stress.

1. Introduction

Rice is the major source of carbohydrate to millions of people world over, particularly in Asia (Price and Tomos, 1997). Rice productivity has been reduced due to global warming that has limited the availability of fresh water for rice crop, particularly under rainfed conditions (Liu et al., 2010). To produce 1 kg of grain, farmers have to supply 2–3 times more water in rice fields than other cereals (Banoc et al., 2000). In Asia, more than 80% of the developed freshwater resources are used for irrigation purposes; about half of which is used for rice production (Asch et al., 2005). Among all the environmental stresses, drought is one of the most severe stresses for rice growth and productivity. Drought stress reduces both nutrient uptake by the roots and transport from roots to the shoots, due to restricted transpiration rates and impaired active transport and membrane permeability (Colmer et al., 1998). Rice is very sensitive to water stress and attempts to reduce water inputs may tax true yield potential (Fukai and Cooper, 1995). The challenge is to develop novel technologies and production systems that would allow rice production to be maintained at the face of declining water availability.

The application of potassium, K can minimize the drought effects of rice. Potassium has substantial effect on enzyme activation, protein synthesis, photosynthesis, stomatal movement and water relations (turgor regulation and osmotic adjustment) in plants (Marschner 1995). Increased application of K has been shown to enhance photosynthetic rate, plant growth, yield, and drought resistance in different crops under water stress conditions (Yadav et al., 1999; Egila et al., 2001; Pervez et al., 2004). K-fed plants maintained higher leaf water potential, turgor potential and rel-

* Corresponding author at: Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang Selangor Darul Ehsan, Malaysia.
E-mail addresses: jae.amalina@gmail.com, amalina86@ummail.um.edu.my (N.A. Mohd Zain), razi@upm.edu.my (M.R. Ismail).

http://dx.doi.org/10.1016/j.agwat.2015.09.022
0378-3774/

ative water content and lower osmotic potential as compared to untreated plants of *Vignaradiata* (Nandwal et al., 1998), maize (Premachandra et al., 1991), and wheat (Pier and Berkowitz, 1987; Sen Gupta et al., 1989) grown under water stress. Nodulation, nitrogenase activity and dry matter yield increased with incremental K supply in broad bean grown at moisture level of only 1/4 of field capacity (Abd-Alla and Wahab, 1995). K is predominant in accumulating solute during drought in tropical grasses (Ford and Wilson, 1981), soybean (Itoh and Kumara, 1987), maize (Premachandra et al., 1991), cotton (Pervez et al., 2004) and olive trees and sunflower (Benlloch-Gonzalez et al., 2008) and significantly contributed to osmotic adjustment.

Under water-deficit conditions, K nutrition increases crop tolerance to water stress by utilizing the soil moisture more efficiently than in K-deficient plants. The positive effects of K on water stress tolerance may be through promotion of root growth accompanied by a greater uptake of nutrients and water by plants (Rama Rao, 1986) and through the reduction of transpirational water loss (Beringer and Trolldenier, 1978). Also, K maintains the osmotic potential and turgor of the cells (Hsio, 1973; Lindhauer, 1985) and regulates the stomatal functioning under water stress conditions (Umar, 2006; Kant and Kafkafi, 2002), which is reflected in improved crop yield in drought conditions (Umar and Bansal, 1997; Umar and Moinuddin, 2002). Besides, it takes part in many essential processes in plants (Marschner, 1995) and enhances photosynthetic rate, plant growth and yield under stress conditions (Egila et al., 2001; Sharma et al., 1996; Tiwari et al., 1998; Umar and Moinuddin, 2002). The protective role of K in plants suffering from drought stress has been attributed to the maintenance of a high pH in stroma and against the photo-oxidative damage to chloroplasts (Cakmak, 1997).

Despite can alleviate water stress effect on rice, potassium are an essential component of plant nutrition and usually used in the form of chloride (KCl), sulfate (K_2SO_4) and nitrate (KNO_3). However, in Malaysia two types potassium are usually found (KCl and K_2SO_4). The used of KCl and K_2SO_4 in reducing the water stress effects was never reported. There was also no recommendation rate of what rates are efficient to tolerate water stress. With that, the objective of present study was (1) to identify the best types (KCl and K_2SO_4) and rates of potassium application (80, 120 and 160 kg K_2O ha^{-1}) in alleviation of water stress in rice. The project was also (2) to investigate the growth and yield, leaf gas exchange and biochemical of rice under cyclic water stress and potassium fertilization. The last objective was (3) to understand the relationship between the yield and leaf gas exchange properties in alleviating water stress effects with potassium fertilization. Understanding the physiological and morphological responses of rice under different cyclic water stress and potassium fertilization is imperative for efficient management of agronomical inputs (irrigation and nutrient) because two of it is important in rice cultivation.

2. Materials and methods

2.1. Experimental location, plant materials and treatments

MR220 rice seeds provided by MARDI Genebank Seberang Perai were germinated and transplanted to tank containing Soil Bakau Series (EC = 2.83 dS m^{-1}; pH 5.1) on 15 days after sown (DAS) in the greenhouse of Ladang 2, Universiti Putra Malaysia, Serdang, Selangor, Malaysia (3°0′34.9020″N latitude; 101°42′7.0416″E longitude; altitude of 46 m above sea level) from December 2009 until April 2010. The light intensity inside the glasshouse was from 225 to 1450 μmol m^{-2} s^{-1}. The relative humidity was between 50 and 60% with a mean atmospheric pressure of 1.013 kPa. The tank with size approximately (55 × 55 × 55) cm had 9 seedlings transplanted in 3 rows × 3 column with space of 20 cm. From 0 DAS until 30 DAS, seedlings were watered with adequate amount of water for 1 cm water level from soil. And on 30 DAS, all tanks were watered at 10 cm water level from soil before first treatment was applied. This factorial experiment was arranged in randomized complete block design (RCBD) replicated four times with two factors i.e. potassium types (KCl and K_2SO_4) and potassium rates in K_2O (80 kg K_2O/ha (control), 120 kg K_2O/ha and 160 kg K_2O/ha). The rice seedlings were exposed to cyclic water stress for 15 days. In this experiment, absolute control was rice irrigated at control flooded + 80 kg K_2O/ha of KCl fertilizer based on standard grower practices in Malaysia. Other fertilizer important like urea (46% N) and triose super phosphate (46% P_2O_5) being added as normal fertilization applied at 120 kg N/ha and 70 kg P_2O_5/ha respectively.

2.2. Measurement

2.2.1. Growth and yield

Rice was harvested when 70% of rice shown ripening colour. Rice panicles with spikelets were cut and collected for every unit before being dried in oven at 60 °C for 2 days. Total panicles for every unit were counted. Then, rice spikelets or grains were separated from the panicle into 2 groups, unfilled grain and filled grain. From here, grain yield, 1000-grains weight and filled spikelets percentage were counted. The rice yield per pot was measured based on grain of 14% moisture content yield weight per pot. Meanwhile for straw biomass was collected from the remaining of plant (without panicle and root) and was dried in a large oven at 70 °C for 3 days. Then the straw was measured for average of 3 hills per unit and being calculated for pot area. Meanwhile harvest index was calculated based on ratio of economic yield to total biomass produced. Water productivity in terms of irrigation was determine by following Molden et al. (2003) as yield (kg ha^{-1})/irrigation inflow (L ha^{-1}). Total plant biomass was taken by calculating the dry weight of root, boles and leaves per seedling. Destructive plant analysis was carried out every 3 weeks for 15 weeks. The plant parts were placed in paper bags and oven dried at 80 °C until constant weight was reached using electronic weighing scale (CDS 125, Mitutoyo Inc., Japan). Leaf area per plant was measured using a leaf area meter (LI-3100, Lincoln Inc., USA). The leaves were arranged within the field of view, and overlapping of adjacent leaves was avoided. Growth analysis was calculated on an individual plant basis through the measurement of total plant leaf area and dry weight. Net assimilation rate (NAR) was calculated based on biomass and leaf area parameters according to the formula reported by Khan et al. (2012).

2.2.2. Leaf gas exchange measurements

The measurement was obtained from a closed infra-red gas analyzer LICOR 6400 Portable Photosynthesis System (IRGA, Licor Inc., Lincoln, NE, USA). The measurements used optimal conditions set of 400 μmol mol^{-1} CO_2, 30 °C cuvette temperature, 60% relative humidity with air flow rate set at 500 cm^3 min^{-1}, and modified cuvette conditions of 225, 500, 625 and 900 μmol m^{-2} respectively, photosynthetically photon flux density (PPFD), according to the irradiance treatment. Gas exchange measurements were carried out between 0900 h and 1100 h, using fully expanded young leaves numbered 3 and 4 from plant apex to record net photosynthesis rate (A). The operation was automatic and the data were stored in the LI-6400 console and analyzed by "Photosyn Assistant" software (version 1.0; Dundee Scientific: Dundee, Scotland, UK, 2000). Several precautions were taken to avoid errors during measurements. Leaf surfaces were cleaned and dried using tissue paper before being enclosed in the leaf cuvette. All measurements were record on fully expanded leaf. Water use efficiency was measured based on the equation by Tanner and Sinchlair (1983):

Table 1
Impact of potassium rates on growth and yield of rice.

Potassium rates ($kg\ K_2O\ ha^{-1}$)	Panicle dry weight $plant^{-1}$ (g)	Root dry weight $plant^{-1}$ (g)	Rice yield ($g\ tank^{-1}$)	Harvest index (HI)
Control*	23.94 ± 3.23a	0.34 ± 0.02b	319.15 ± 22.97a	0.52 ± 0.12a
80	12.31 ± 1.25d	0.31 ± 0.12b	129.43 ± 25.81c	0.30 ± 0.22d
120	21.62 ± 0.65b	0.31 ± 0.08b	211.40 ± 23.26b	0.42 ± 0.42b
160	18.29 ± 1.27c	0.45 ± 0.06a	191.19 ± 44.48b	0.40 ± 0.31c
Main effects				
K Rates	**	**	**	**
K Types	ns	ns	ns	ns
K R × K T	ns	ns	ns	ns

All analyses are mean ± standard error of means (SEM), *control = 80 kg K_2O ha^{-1} KCl fertilizer+ control flooded, ** = significant at 1% level, ns = no significant ($P < 0.05$).

WUE = $\frac{P_n}{E_p}$ P_n = net Co2 assimilation rate (μmol/m²/s) and E_p is evapo-transpiration rate (mmol/m²/s).

2.2.3. Assay of Catalase (CAT) activity

2.2.3.1. Preparation of enzyme extracts. To determine the enzymatic activities of the antioxidant proteins, a crude enzyme extracts was prepared by homogenizing 500 mg of leaf tissue in extraction buffer containing 0.5% Triton X-100 and 1% polyvinylpyrrolidone in 100 mM potassium phosphate buffer (pH 7.0) using a chilled mortar and pestle. The homogenate was centrifuged at 15,000 rpm for 20 min at 4 °C. The supernatant was used for the enzymatic assays. Catalase activity (CAT; EC 1.11.1.6) was determined by consumption of H_2O_2 using the method of Aebi (1983). The reaction mixture (3 mL) contained 50 mM potassium phosphate buffer pH 7.0, 15 mM H_2O_2 and 50 μL enzyme extract. The reaction was initiated by adding the H_2O_2. The consumption of H_2O_2 was monitored spectrophotometrically at 240 nm for 3 min. Enzyme activity was expressed in micromole per liter H_2O_2 min^{-1}.

2.2.4. Malondialdehyde (MDA content)

1 g of fresh leaves was ground in 5 mL of 0.5% trichloroacetic acid (TCA) and centrifuged at 1000× g for 15 min. The mixture containing 1 mL of the supernatant and 4 mL of 0.5% thiobarbituricacid (TBA) in 20% TCA was heated at 100 °C for 30 min and then cooled to room temperature. The specific absorbance (at 532 nm) of the extract (relative to the background absorbance at 600 nm) was determined. The concentration of malondialdehyde (MDA) was expressed in μmol g^{-1} FW (fresh weight of leaves), using a molar extinction coefficient equal to 155 × 105 $mmol^{-1}$ cm^{-1}.

2.2.5. Proline determination

About 5 mg of lyophilized and homogenized samples were extracted in 0.5 mL of 3% 5-sulphosalicylic acid for 15 min. Then the samples were centrifuged at 21,000 × g for 15 min. The clear supernatant (200 μL) was transferred to polypropylene screw cap vials, after which 200 μL of concentrated formic acid and 400 μL of 3% ninhydrin reagent in 2-methoxyethanol were added. Samples were heated for 0.5 h at 100 °C in a water-bath, and then transferred to 96-well plates. Absorbance was measured at 514 nm on a micro-plate reader (Synergy 2, Bio-Tek, Winooski, VT, USA). The level of proline was expressed in μg g^{-1} DW (dry weight of leaves).

2.3. Statistical analysis

Data were analyzed using Proc GLM using SAS 9.2 (32). Mean separation test between treatments was performed using Least Significant Different, LSD and standard error of differences between means was calculated with the assumption that data were normally distributed and equally replicated.

3. Result and discussion

Overall results showed that half of parameters were influenced by interaction effects of potassium rates and types. There were shoot dry weight, leaf area, total spikelet $panicle^{-1}$, total empty spikelet $panicle^{-1}$, biomass rate, net assimilation rate, transpiration rate and water use efficiency of rice. Based on results, potassium types only had significant different on total tillers $plant^{-1}$, leaves number $plant^{-1}$ and 1000 grains weight of rice. This showed that the companion nutrient (chloride and sulphate) in potassium fertilizer had only affected on tiller and leaves growth and grains weight. Meanwhile potassium rate had more influenced on catalase activity, free proline, Maliondialdehyde content, panicle dry weight $hill^{-1}$, root dry weight $hill^{-1}$, rice yield and harvest index.

3.1. Panicle dry weight hill^{-1}, root dry weight hill^{-1}, rice yield and harvest index

It was observed that panicle dry weight $hill^{-1}$, root dry weight $hill^{-1}$, rice yield and harvest index was influenced by potassium rates ($P < 0.05$; Table 1). The panicle dry weight $hill^{-1}$ was highest in control that record 23.94 followed by 120 kg K_2O ha^{-1} (21.62), 160 kg K_2O ha^{-1} (18.29) and 80 kg K_2O ha^{-1} (12.31). There was no significant in root dry weight $hill^{-1}$ in control, 80 kg K_2O ha^{-1} and 120 kg K_2O ha^{-1} potassium application compared to the application of 160 kg K_2O ha^{-1} that gave the highest root dry weight (0.45 g). Table 1 had showed that rice yield was lowest under 80 kg K_2O ha^{-1} that record 129.43 g $tank^{-1}$ compared to control (319.15 g $tank^{-1}$), followed by 120 kg K_2O ha^{-1} (211.40 g $tank^{-1}$) and 160 kg K_2O ha^{-1} (191.19 g $tank^{-1}$). The result suggested that increasing of potassium fertilizer rate during cyclic water stress 15 days reduced the yield difference to control. The same attributes to harvest index when the highest harvest index was obtained in control (0.52), followed by 120 (0.42), 160 (0.40) and 80 kg K_2O ha^{-1} (0.30). Rice yield and harvest index had a significant correlation with leaf area ($r = 0.921$; $p \leq 0.05$; Table 2). This included more leaf area needed by this variety to obtained higher yield and harvest index. This indicates as the rate of potassium take increases, there will be increases of leaf area that simultaneously increased the yield and harvest index of rice. The same result was observed as the rate of potassium increase in Sorghum the yield and harvest index was significantly increased (Shahid, 2006).

3.2. Leaves number, total tillers and 1000 grains weight

The leaves number, total tillers and 1000 grains weight was influenced by the potassium types ($p \leq 0.05$; Fig. 1). The leaves number was statistically high in K_2SO_4 (17.14) but control (14.89) had no significant different with KCl (14.80) application. In total tillers, the application of K_2SO_4 produced highest tillers number (5.57) compared to KCl (4.17) and control (4.02). Both result on

Table 2
Pearsons correlations between all parameters measured in the study.

Parameters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
1	PDW/Hill	1.000															
2	RDW	0.213	1.000														
3	S/P	0.432	0.234	1.000													
4	Yield	0.097	0.256	0.231	1.000												
5	HI	0.453	0.432	0.235	0.008	1.000											
6	LeafNo	0.234	0.123	0.156	0.921*	0.124	1.000										
7	Tillers	0.211	0.111	0.187	0.871*	0.432	0.123	1.000									
8	1000gw	0.324	0.009	0.165	0.971*	0.097	0.009	0.234	1.000								
9	Area	0.546	0.082	0.176	0.921*	0.923*	0.082	0.134	0.324	1.000							
10	SDW/H	0.123	0.126	0.234	0.124	0.971*	0.126	0.156	0.009	0.982*	1.000						
11	NAR	0.009	0.231	0.134	0.912*	0.211	0.231	0.008	0.165	0.924*	0.234	1.000					
12	CAT	0.128	0.453	0.156	0.223	0.134	0.009	0.007	0.971*	0.265	0.123	0.167	1.000				
13	Proline	0.265	0.213	0.008	0.432	0.097	0.453	0.234	0.211	0.324	0.546	0.123	0.911*	1.000			
14	MDA	0.123	0.124	0.007	0.187	0.165	0.176	0.234	0.134	0.156	0.008	0.007	-0.871*	0.715*	1.000		
15	Transpiration	0.167	0.156	0.124	0.432	0.097	0.453	0.234	0.211	0.324	0.546	0.897*	0.871*	0.076	0.213	1.000	
16	WUE	0.098	0.345	0.234	0.807*	0.912*	0.324	0.546	0.123	0.009	0.128	0.265	0.123	0.167	0.098	0.321	1.000

*Significant at $P \leq 0.05$. Note, PDW/hill = panicle dry weight hill^{-1}; RDW = root dry weight hill^{-1}; S/P = spikelets panicle^{-1}; HI = harvest index; LeafNo = leaf numbers hill^{-1}; 1000gw = 1000 grains weight; Area = leaf area; SDW/H = shoot dry weight hill^{-1}; NAR = net assimilation rate; CAT = catalase; MDA = Maliondialdehyde; WUE = instantaneous water use efficiency, ** = significant at 1% level, * = significant at 5% level.

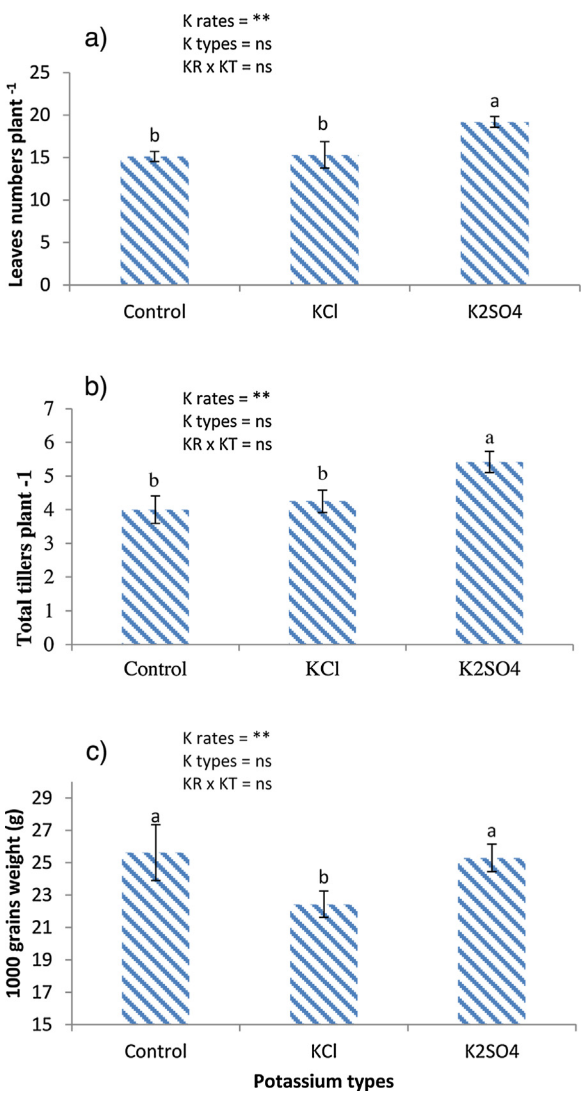

Fig. 1. Impact of potassium types on leaves numbers plant^{-1} (a), total tillers plant^{-1} (b) and 1000 grains weight (c) of rice. $N = 28$. Bars represent standard error of differences between means. Control = 80 kg K_2O ha^{-1} KCl + control flooded, ** = significant at 1% level, ns = no significant ($p < 0.05$).

leaves number and total tillers showed that K_2SO_4 produced highest level greater than control. There were no significant different in 1000 grains weight between control (25.01) and K_2SO_4 (25.14), however KCl record the lowest 1000 grains weight (23.12). The increase in the vegetative growth of rice by application of K_2SO_4 was observed by Hara and Khan et al. (2012). The leaves number, total tillers and 1000 grains weight have a significant positive correlation with rice yield (Leaves number, $r = 0.92$, tillers, $r = 0.87$ and 1000 grains weight, $r = 0.97$; $p \leq 0.05$; Table 2). The data indicates that the increases in the leaves number, total tillers and 1000 grains weight were simultaneously increase the rice yield. This showed the importance of these components influence the rice yield.

3.3. Leaf area and shoot dry weight

The leaf area and shoot dry weight was influenced by interaction effects between potassium rate and types ($p \leq 0.05$; Fig. 2). The leaf area and shoot dry weight showed almost the same trends where the highest leaf area was in shoot dry weight was in K_2SO_4 + 120 kg K_2O ha^{-1} that recorded 181.35 cm^2 and 1.64 g respectively. The lowest in shoot dry weight was observed in K_2SO_4 + 80 kg K_2O ha^{-1} that record 0.66 g meanwhile the highest was showed in K_2SO_4 + 120 kg K_2O ha^{-1} that 2 fold greater than control respectively. And K_2SO_4 + 120 kg K_2O ha^{-1} also had highest level followed by KCl + 80 kg K_2O ha^{-1}. The current study suggested that application of K_2SO_4 at 120 kg K_2O ha^{-1} can enhance the leaf area and shoot biomass in rice. High significant correlation between leaf area and shoot dry weight ($r = 0.98$; $p \leq 0.05$; Table 2) suggested that a lot of energy from light reaction being invested to the above ground biomass production.

3.4. Total spikelet panicle^{-1} and empty spikelet panicle^{-1}

The interaction between potassium rates and types has influenced total spikelet panicle^{-1} and empty spikelet panicle^{-1} ($p \leq 0.05$; Fig. 3). Total spikelet panicle^{-1} in KCl was highest in application of 120 kg K_2O ha^{-1} (214.54) and at K_2SO_4 + 80 kg K_2O ha^{-1} the total spikelet/panicle was optimized (193.66). The application of KCl + 80 kg K_2O ha^{-1} (168.25) record the lowest total spikelet/panicle compared to the control (186.01) with the difference was only 9.5%. Interestingly, it was observed that the empty spikelet panicle^{-1} was enhanced in all combination treatments compared to the control. The highest empty spikelet panicle^{-1} was observed K_2SO_4 + 80 kg K_2O ha^{-1} (112.33) and lowest in application of K_2SO_4 + 120 kg K_2O ha^{-1} that recorded 66.16. The result showed

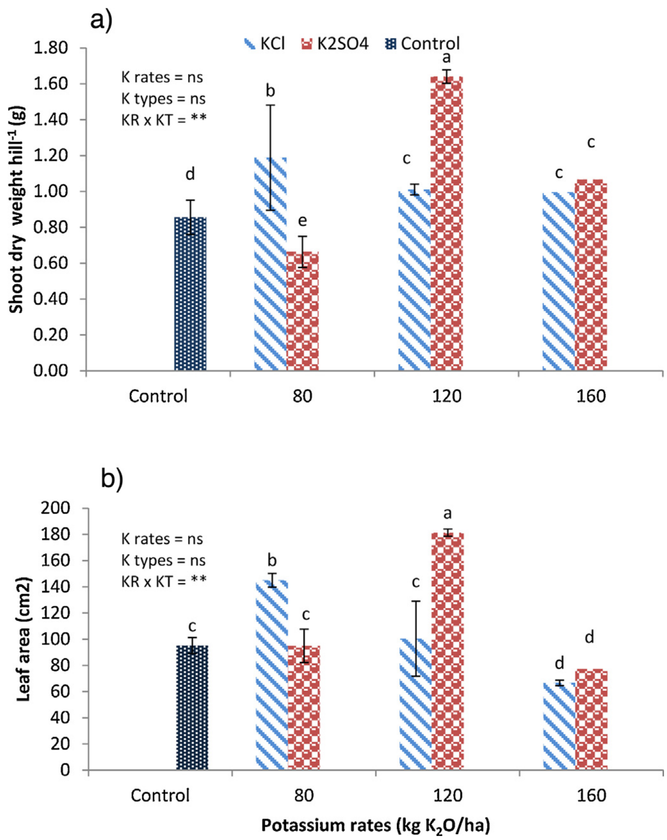

Fig. 2. Interaction effects between potassium rates and sources on shoot dry weight hill^{-1} (a) and leaf area (b) of rice. N = 28. Bars represent standard error of differences between means. Control = 80 kg K_2O ha^{-1} KCl + control flooded, ** = significant at 1% level, ns = no significant ($p < 0.05$).

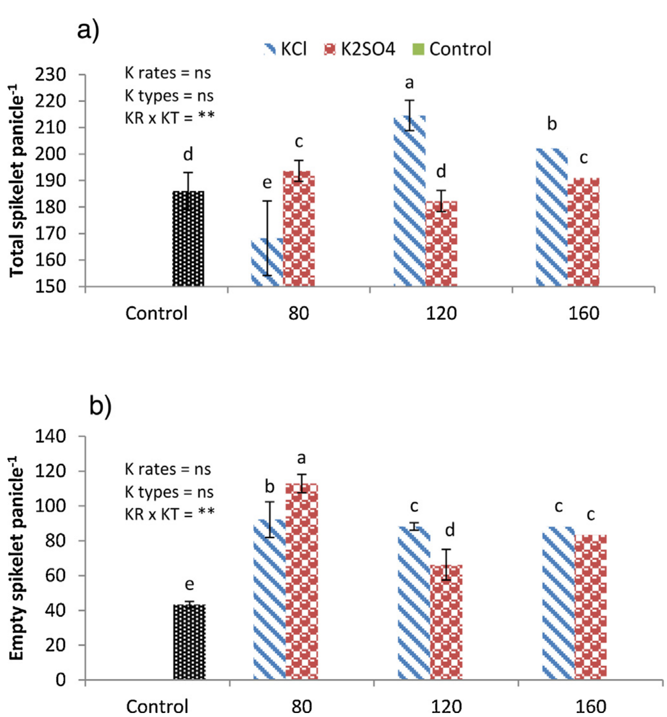

Fig. 3. Interaction effects between potassium rates and sources on (a) total spikelet panicle^{-1} and (b) total empty spikelet panicle^{-1}. N = 28. Bars represent standard error of differences between means. Control = 80 kg K_2O ha^{-1} KCl + control flooded, ** = significant at 1% level, ns = no significant ($p < 0.05$).

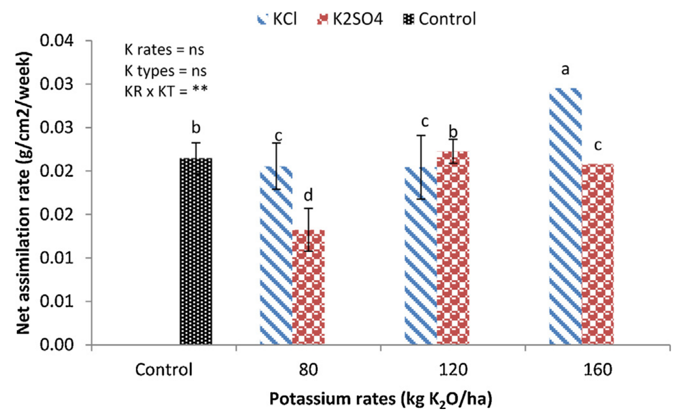

Fig. 4. Interaction effects between potassium rates and sources on net assimilation rates of rice. N = 28. Bars represent standard error of differences between means. Control = 80 kg K_2O ha^{-1} KCl + control flooded, ** = significant at 1% level, ns = no significant ($p < 0.05$).

that increasing potassium rate reduce percentage of empty spikelet, and the present study showed that reduction in yield of rice under influence of water stress in rice might be contributed to increase in empty spikelet panicle^{-1}. The increased in incidence of empty spikelet panicle^{-1} under water stress was never reported, however study by Rad et al. (2012) and Aref and Rad (2012) have showed that increased in salinity stress have increased the incidence of empty spikelet panicle^{-1} in rice. This concludes that drought stress might reduce the rice yield by increasing the increase in empty spikelet panicle^{-1}.

3.5. Net assimilation rate

Interaction effects between potassium rate and types have influenced the net assimilation rate (NAR) ($p \leq 0.05$; Fig. 4). The highest NAR was recorded at KCl + 160 kg K_2O ha^{-1} that recorded 0.029 g cm^{-2} week^{-1}, while the lowest at K_2SO_4 + 80 kg K_2O ha^{-1} that just recorded 0.013 g cm^{-2} week^{-1}. The highest NAR under application K_2SO_4 was at 120 kg K_2O ha^{-1} (0.022 g cm^{-2} week^{-1}). This indicated that addition KCl can enhance NAR better than addition of K_2SO_4. The higher NAR with KCl compared to K_2SO_4 might be due to higher absorption of K element in rice leaves by KCl compared to K_2SO_4. This was in agreement with the findings of Khadr et al. (2004) that observed that application of KCl improved the K uptake in sugar cane compared to the use of K_2SO_4 by 3%. This suggested that K was more available to the plant in KCl compared to K_2SO_4. Correlation table showed that NAR have a significant positive correlation with transpiration (r = 0.89; $p \leq 0.05$; Table 2) and rice yield (r = 0.912; $p \leq 0.05$; Table 2). This suggests that increases in transpiration rate under high application of potassium might enhanced photosynthesis rate and simultaneously enhanced the rice yield. High significant positive correlation was also observed between NAR and leaf area (r = 0.92; $p \leq 0.05$; Table 2) indicating enhancement of growth of rice are mostly influenced by increase in light absorption area compared to the other growth factors. Similar result was also obtained by Zeng et al. (2010) in rice where they observed linear correlation between NAR and leaf area.

3.6. Catalase activity

From the present study it was observed that CAT activity was influenced by potassium rate ($p \leq 0.05$; Fig. 5). The CAT activity was reduced to 40% and 12% respectively in 160 kg K_2O ha^{-1} and 120 kg K_2O ha^{-1} potassium rate compared to the control however, application of 80 kg K_2O ha^{-1} potassium increased the CAT activity 22% higher than the control treatment. The data indicate that

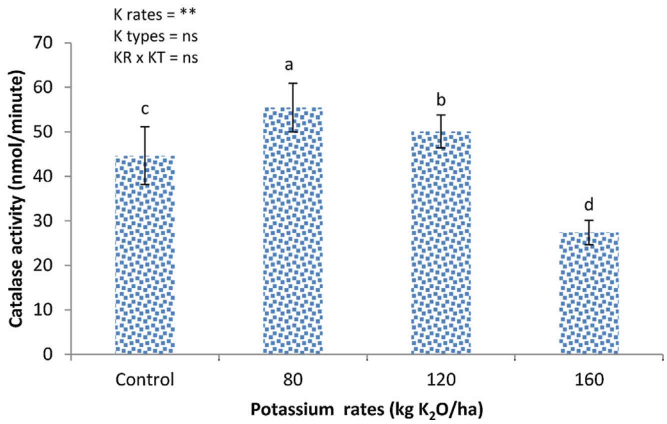

Fig. 5. Impact of potassium rates on catalase activity of rice. $N=28$. Bars represent standard error of differences between means. Control = 80 kg K_2O ha^{-1} KCl + control flooded, ** = significant at 1% level, ns = no significant($p<0.05$).

application of high potassium would reduce the CAT activities. Catalase functions as effective quenchers for ROS (Foyer et al., 1994). CAT plays an essential role in scavenging from H_2O_2 toxicity. This showed that oxidative stress can be reduced by high application of potassium in rice. These result also implied that fertilization with high potassium can play important protective role in H_2O_2 scavenging processes. It was reported by Tripathi et al. (2009) that proteins such as thioredoxin, glutaredoxin and cyclophilin are known to facilitate the regeneration of the reduced (catalytically active) form of peroxyredoxin that plays an important role in reducing the ROS formation in plants under biotic and abiotic stress, the increase application of potassium fertilizer would increase these proteins (Chaitanya et al., 2002). The present study was in agreement with Ibrahim and Jaafar (2012) on *Labisia pumila* where application of potassium from 90 > 270 kg ha^{-1} have reduced the CAT activity and indicate low oxidative stress of the plants. It also was observed from the correlation table that CAT have a significant negative relationship with transpiration rate ($r=-0.871$; $p\leq0.05$; Table 2) that indicate reduction in oxidative stress might be due to increase in transpiration rate under high potassium fertilization, this suggest that enhancement of transpiration rate might reduce the water stress effects in rice.

3.7. Proline and MDA

It was observed that potassium levels have influenced the proline and lipid peroxidation of the rice ($p\leq0.05$; Fig. 6). Generally as potassium levels increased, the production of proline has shown to increased. Proline content was enhanced by 152, 257 and 338% as compared to control that just recorded 2.1 mg g^{-1} fresh weight. The lipid peroxidation was highest under 160 kg K_2O ha^{-1} that recorded 6.4 mg g^{-1} followed by 120 kg K_2O ha^{-1} (5.2 mg g^{-1}), 80 kg K_2O ha^{-1} (4.2 mg g^{-1}) and Control at 3.1 mg g^{-1} (Fig. 6). In Fig. 7 it was shown that the increased in proline content have increased the production in catalase and lipid peroxidation in rice, where proline have a significant positive regression with Catalase ($r=0.910$; $p\leq0.05$) and lipid peroxidation ($r=0.715$; $p\leq0.05$; Table 2). The same observation was also obtained in *Vacia faba* (Siddiqui et al., 2012) and *Halocnemum strobilaceum* (Su et al., 2012) under cadmium and salt stress. This result indicates that under application of high potassium to rice, stress can occurred and be manisfested by steady increase in proline, and maliondialdehyde content.

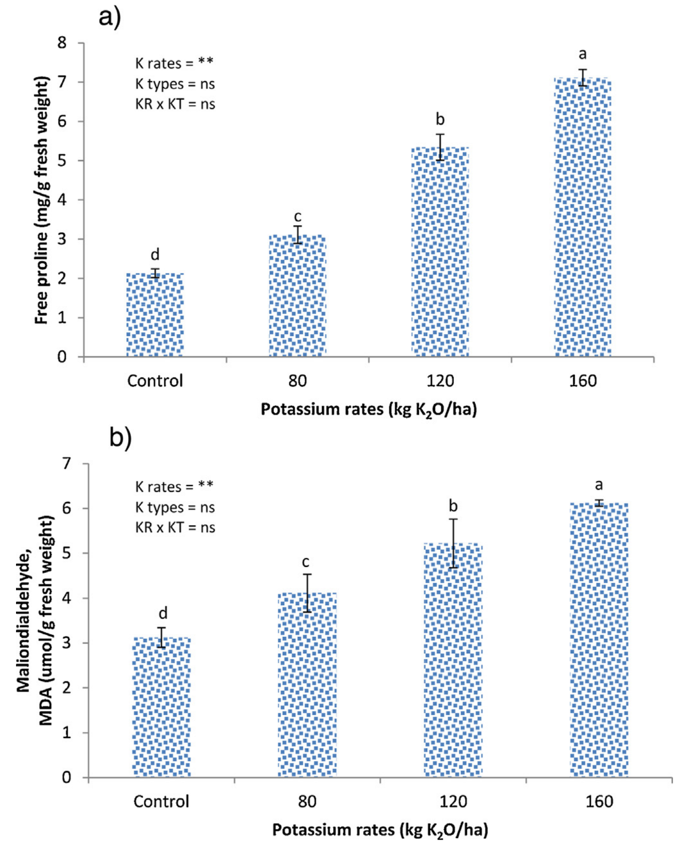

Fig. 6. Impact of potassium rates on free proline (a) and Maliondialdehyde content (b) of rice. $N=28$. Bars represent standard error of differences between means. Control = 80 kg K_2O ha^{-1} KCl + control flooded, ** = significant at 1% level, ns = no significant ($p<0.05$).

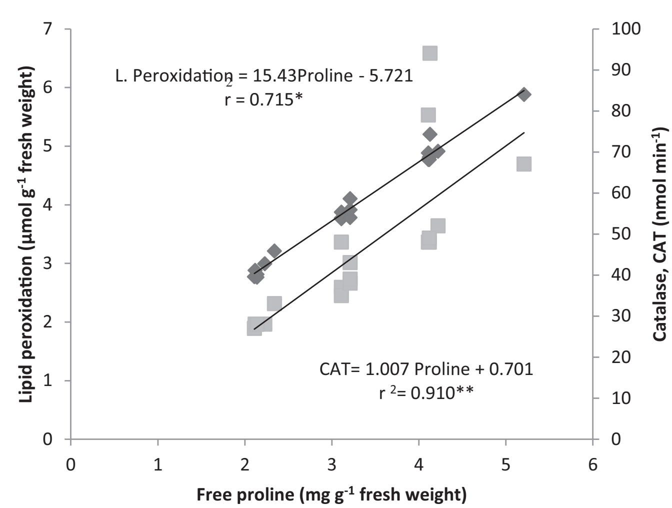

Fig. 7. Relationship between free proline content, lipid peroxidation and catalase activity. $N=28$. * and ** significant at $p\leq0.05$ and $p\leq0.01$ respectively.

3.8. Transpiration rate and water use efficiency

Transpiration rate was enhanced by interaction effects between potassium rate and types ($p\leq0.05$; Fig. 8). Generally, at 80 kg K_2O ha^{-1} potassium, transpiration rate in KCl (8.52 mmol m^{-2} s^{-1}) and K_2SO_4 (9.01 mmol m^{-2} s^{-1}) was the lowest compared to the control that recorded 9.66 mmol m^{-2} s^{-1}. As the potassium rate increased from 80 > 120 > 160 kg K_2O ha^{-1} the transpiration rate was observed to be increased in both

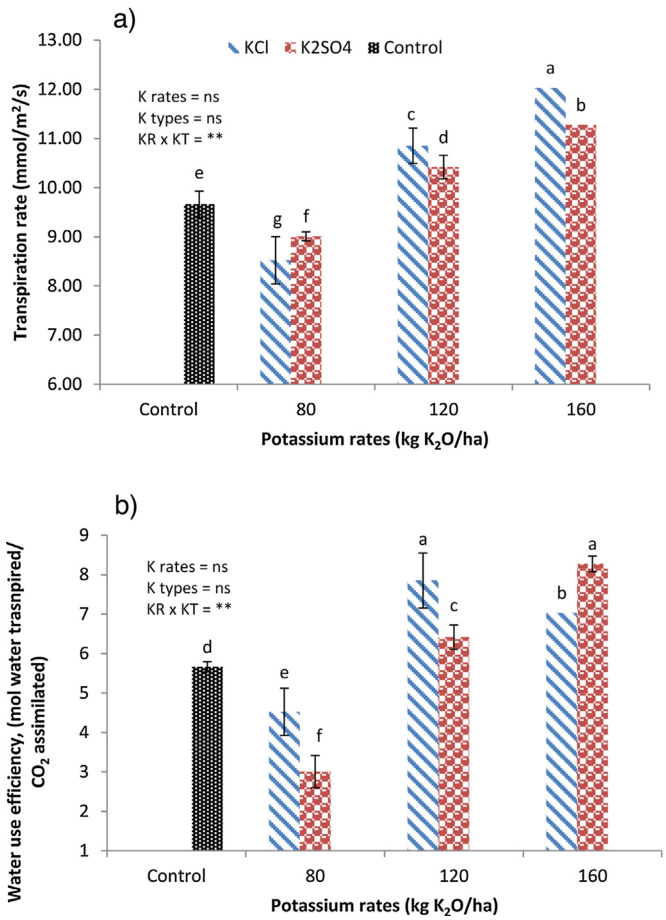

Fig. 8. Interaction effects between potassium rates and sources on transpiration rates (a) and water use efficiency (b) of rice. $N = 28$. Bars represent standard error of differences between means. Control = 80 kg K_2O ha^{-1} KCl + control flooded. ** = significant at 1% level, ns = no significant ($p < 0.05$).

KCl and K_2SO_4, with the highest transpiration rate recorded in KCl + 160 kg K_2O ha^{-1} (12.03 mmol m^{-2} s^{-1}). The result suggested that high potassium rates would reduce water stress effects by having high transpiration rate. High transpiration rate would increase the nutrient uptake that would repair the damage tissue under water stress thus reduce the oxidative stress of rice under water stress condition. This been showed by high significant negative correlations of transpiration rate with CAT activity ($r = -0.871$; $p \le 0.05$; Table 2). The increased in transpiration rate with increased in potassium fertilization have been observed by the other researches (Xu et al., 2011; Kanai et al., 2011; Sritontip et al., 2008). In 80 and 120 kg K_2O ha^{-1}, it was found that the water use efficiency was highest under application of KCl than K_2SO_4 that recorded 4.52 and 7.85 in 80 and 120 kg K_2O ha^{-1} respectively compared to 3.01 and 6.41 in 80 and 120 kg K_2O ha^{-1} respectively in K_2SO_4. At 160 kg K_2O ha^{-1}, the application of K_2SO_4 gave the highest WUE (8.27) compared to KCl that registered 7.03. The control treatment just recorded 5.66. The current study suggests that application of potassium at higher rates can improve the water use efficiency in rice. The increase in water use efficiency in rice might be contributed by efficient turgor regulation and osmotic adjustment under high potassium application in rice (Marshner, 1995). The water use efficiency have a significant correlationship with rice yield ($r = 0.887$; $p \le 0.05$; Table 2) and Harvest index ($r = 0.910$; $p \le 0.05$; Table 2) that indicate the alleviation of drought stress in rice under potassium fertilization might be due to increase in water use efficiency. This was supported by previous studies that showed increased application of potassium would increase the efficiency of plant to use water.

4. Conclusion

The application of potassium fertilizers can increase the tolerance of rice under cyclic water stress. The application of potassium at 120 kg K_2O ha^{-1} either KCl or K_2SO_4 fertilizer was found to be efficient to minimize reduction in rice yield and harvest index under cyclic water stress 15 days and possible to substitute control flooded practice during water scarcity. It was found potassium might tolerate to cyclic water stress by increase its transpiration rate, net assimilation rate, proline and maliondialdehyde and reduce catalase activity as indication of low oxidative stress under these condition.

Acknowledgements

The authors are grateful to the Ministry of Higher Education Malaysia and Long-term Research Grant Scheme (LRGS) for financing this work under the Research University Grant.

References

Abd-Alla, M.H., Wahab, A.M.A., 1995. Response of nitrogen fixation, nodule activities, and growth to potassium supply in water-stressed broad bean. J. Plant Nutr. 18, 1391–1402.
Aebi, H., 1983. Catalase. In: Bergmeyer, H., Verlag, C., Weinheim, A. (Eds.), Methods of Enzymatic Analysis. , 3rd ed. Verlag Chemie, Weinheim, Germany, pp. 273–277.
Aref, F., Rad, H.E., 2012. Physiological characterization of rice under salinity stress during vegetative and reproductive stages. Indian J. Sci. Technol. 5, 2578–2586.
Asch, F., Dingkuhn, M., Sow, A., Audebert, A., 2005. Drought-induced changes in rooting patterns and assimilate partitioning between root and shoot in upland rice. Field Crops Res. 93, 223–236.
Banoc, D.M., Yamauchi, A., Kamoshita, A., Wade, L.J., Pardales, J.R., 2000. Genotypic variations in response of lateral root development to fluctuating soil moisture in rice. Plant Prod. Sci. 3, 335–343.
Benlloch-Gonzalez, M., Arquero, O., Fournier, J.M., Barranco, D., Benlloch, M., 2008. K+ starvation inhibits water-stress-induced stomatal closure. Plant Physiol. 165, 623–630.
Beringer, H., Trolldenier, G., 1978. Influence of K nutrition on the response to environmental stresses. In: Potassium Research—Reviews and Trends. International Potash Institute, Basel, Switzerland, pp. 189–222.
Cakmak, I., 1997. Role of potassium in protecting higher plants against photo-oxidative damage. In: Johnston, A.E. (Ed.), Food Security in the WANA Region, the Essential Need for Balanced Fertilization. International Potash Institute, Basel, Switzerland, pp. 345–352.
Chaitanya, K., Sundar, D., Masilamani, S., Ramachandra, R.A., 2002. Variation in heat stress induced antioxidant enzyme activities among three mulberry cultivars. Plant Growth Regul. 36, 175–180.
Colmer, T.D., Gibberd, M.R., Wiengweera, A., Tinh, T.K., 1998. The barrier to radial oxygen loss from roots of rice (*Oryza sativa* L.) is induced by growth in stagnant solution. Exp. Bot. 49, 1431–1436.
Egila, J.N., Davies Jr., F.T., Drew, M.C., 2001. Effect of potassium on drought resistance of *Hibiscus rosa-sinensis* cv. Leprechaun: plant growth, leaf macro and micronutrient content and root longevity. Plant Soil 229, 213–224.
Ford, C.W., Wilson, J.R., 1981. Changes in levels of solutes during osmotic adjustment to water stress in leaves of four tropical pasture species. Aust. J. Plant Physiol. 8, 77–91.
Foyer, C.H., Decourviers, P., Kenerk, K.J., 1994. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. Plant Cell Environ. 17, 507–523.
Fukai, S., Cooper, M., 1995. Development of drought-resistant cultivarsusing physio-morphological traits in rice. Field Crops Res. 40, 67–86.
Hsio, T.C., 1973. Plant responses to water stress. Ann. Rev. Plant Physiol. 24, 519–570.
Ibrahim, M.H., Jaafar, H.Z.E., 2012. Primary, secondary metabolites, H_2O_2, malondialdehyde and photosynthetic responses of *Orthosiphon stamineus* Benth. to different irradiance levels. Molecules 17, 1159–1176.
Itoh, R., Kumara, A., 1987. Acclimation of soybean plants to water deficit. V. Contribution of potassium and sugar to osmotic concentration in leaves. Jpn. J. Crop Sci. 56, 678–684.
Kanai, S., Moghaieb, R.E., El-Shemy, H.A., Panigrahi, R., Mohapatra, P.K., Ito, J., Nguyen, N.T., Saneoka, H., Fujita, K., 2011. Potassium deficiency affects water status and photosynthetic rate of the vegetative sink in green house tomato prior to its effects on source activity. Plant Sci. 180, 368–374.

Kant, S., Kafkafi, U., 2002. Potassium and Abiotic Stresses in Plants. In: Pasricha, N.S., Bansal, S.K. (Eds.), Potassium for Sustainable Crop Production. Potash Institute of India, Gurgaon, India, pp. 233–251.

Khadr, M.S., Negm, A.Y., Khalil, F.A., Antoun, L.W., 2004. Effect of potassium chloride in comparison with potassium sulfate on sugar cane production and some soil chemical properties under Egyptian conditions. Rabat, Morocco, 24–28 November In: IPI Regional Workshop on Potassium and Fertigation development in West Asia and North Africa, 004, pp. 1–8.

Khan, A.W., Mann, R.A., Saleem, M., Majeed, A., 2012. Comparative rice yield and economic advantage of foliar KNO_3 over soil applied K_2SO_4. Pak. J. Agric. Sci. 49, 481–484.

Lindhauer, M.G., 1985. Influence of K nutrition and drought and water stressed sunflower plants differing in K nutrition. J. Plant Nutr 10, 1965–1973.

Liu, S.P., Zheng, L.Q., Xue, Y.H., Zhang, Q.A., Wang, L., Shou, H.X., 2010. Overexpression of OsVP1 and OsNHX1 increases tolerance to drought and salinity in rice. Plant Biol. 53, 444–452.

Marschner, H., 1995. Mineral Nutrition of Higher Plants, 2nd ed. Academic Press, San Diego, California, USA.

Molden, D., Murray-Rust, H., Sakthivadivel, R., Makin, I., 2003. A water-productivity framework for understanding and action. Water productivity in agriculture: limits and opportunities for improvement. In: Kijne, J.W., Barker, R., Molden, D. (Eds.), Comprehensive Assessment of Water Management in Agriculture Series, No. 1. International Water Management Institute, Sri Lanka.

Nandwal, A.S., Hooda, A., Datta, D., 1998. Effect of substrate moisture and potassium on water relations and C, N and K distribution in Vignaradiata. Biol. Plant. 41, 149–153.

Pervez, H., Ashraf, M., Makhdum, M.I., 2004. Influence of potassium nutrition on gas exchange characteristics and water relations in cotton (*Gossypium hirsutum* L.). Photosynthetica 42, 251–255.

Pier, P.A., Berkowitz, G.A., 1987. Modulation of water stress effects on photosynthesis by altered leaf K^+. Plant Physiol. 85, 65–661.

Premachandra, G.S., Saneoka, H., Ogata, S., 1991. Cell membrane stability and leaf water relations as affected by potassium nutrition of water-stressed maize. Exp. Bot. 42, 739–745.

Price, A.H., Tomos, A.D., 1997. Genetic dissection of root growth in rice (*Oryza sativa* L.). II. Mapping quantitative trait loci using molecular markers. Theor. Appl. Gene 95, 143–152.

Rad, H.E., Aref, F., Rezaei, M., 2012. Rice growth and yield components respond to changes in water salinity stress. World Appl. Sci. J. 20, 997–1007.

Rama Rao, N., 1986. Potassium nutrition of pearl millet subjected to moisture stress., J. Potassium Res. 2, 1–12.

Sen Gupta, A., Berkowitz, G.A., Pier, P.A., 1989. Maintenance of photosynthesis at low leaf water potential in wheat. Plant Physiol. 89, 1358–1365.

Shahid, U., 2006. Alleviating adverse effects of water stress on yield of sorghum: mustard and groundnut by potassium application. Pak. J. Bot. 38, 1373–1380.

Sharma, K.D., Nandwal, A.S., Kuhad, M.S., 1996. Potassium effects on CO_2 exchange, NRA and yield of cluster bean cultivars under water stress. J. Potassium Res. 12, 412–423.

Siddiqui, M.H., Al-Whaibi, M.H., Sakran, A.M., Basalah, M.O., Ali, H.M., 2012. Effect of calcium and potassium on antioxidant system of *Vicia faba* L. under cadmium stress. Int. J. Mol. Sci. 13, 6604–6619.

Sritontip, C., Khaosumain, Y., Changjeraja, S., Changjeraja, R., 2008. Effects of light intensity and potassium chlorate on photosynthesis and flowering in 'Do' longan. Acta Hortic. 787, 285–288.

Su, D., Chen, N., Gao, T., Wang, C., Sheng, M., Yang, C., 2012. Effects of Si^+, K^+, and Ca^{2+} on antioxidant enzyme activities and osmolytes in *Halocnemum strobilaceum* under salt stress. Adv. Mater. Res. 356–360, 2542–2550.

Tanner, C.B., Sinchlair, T.R., 1983. Efficient water use in crop production reaseach. In: Taylor, H.M., Jordon, W.R., Sinchlair, T.R. (Eds.), Limitation to Efficient Water Use in Crop Production. ASA/CSAA/ASSA, Wisconsin, pp. 1–8.

Tiwari, H.S., Agarval, R.M., Bhatt, R.K., 1998. Photosynthesis, stomatal resistance and related characters as influenced by potassium under normal water supply and water stress condition in rice (*Oryza sativa* L.). Indian J. Plant Physiol. 3, 314–316.

Tripathi, B.N., Bhatt, I., Dietz, K.J., 2009. Photosynthetic organism. Protoplas 235, 3–15.

Umar, S., 2006. Alleviating adverse effects of water stress on yield of sorghum, mustard and groundnut by potassium application. Pakistan J. Bot. 38, 1373–1380.

Umar, S., Bansal, S.K., 1997. Effect of potassium application on water stressed groundnut. Fert. News 42, 27–29.

Umar, S., Moinuddin, 2002. Genotypic differences in yield and quality of groundnut as affected by potassium nutrition under erratic rainfall conditions. J. Plant Nutr 25, 1549–1562.

Xu, Y.W., Zou, Y.T., Husaini, A.M., Zeng, J.W., Guan, L.L., Liu, Q., Wu, W., 2011. Optimization of potassium for proper growth and physiological response of *Houttuynia cordata* Thunb. Environ. Exp. Bot. 71, 292–297.

Yadav, D.S., Goyal, A.K., Vats, B.K., 1999. Effect of potassium in *Eleusine coracana* (L.) Gaertn. Under moisture stress conditions. J. Potas. Res. 15, 131–134.

Zeng, Q., Liu, B., Gilna, B., Zhang, Y., Zhu, C., Ma, H., Pang, J., Chen, G., Zhu, J., 2010. Elevated CO_2 effects on nutrient competition between a C3 crop (*Oryza sativa* L.) and a C4 weed (*Echinochloacrus-galli* L.). Nutr. Cycl. Agroecosyst. 89, 93–104.