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Full Length Research Paper

Impact of regulated deficit irrigation on the physiological characteristics of two rapeseed varieties as affected by different potassium rates

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A field trial was conducted at experimental field of Qazvin region, Iran (36° 18´ N and 49° 57´ E) in 2008/2009 and 2009/2010 growing seasons to determine the physiological properties of rapeseed varieties (Zarfam and Opera) subjected to drought stress condition under different potassium sulphate levels. The trial was laid out in a factorial experiment based on a randomized complete block design with three replications. Irrigation regimes included I_1, irrigation after 40% (control); I_2, irrigation after 60% and I_3, irrigation after 80% soil water depletion (SWD). However, the applied potassium rates were K_1, non-application, K_2, 100 kg ha^{-1} and K_3, 200 kg ha^{-1} of K_2SO_4. Physiological indices including relative water content (RWC), stomatal resistance (RS), canopy temperature (T_c), and the difference between canopy and air temperature (ΔT) were measured at three stages. Both varieties regarding physiological indices in all sampling stages showed a significant difference. Higher relative water content (RWC) and lower stomatal resistance (RS) and canopy temperature (T_c) in Opera variety under limited moisture condition indicated its salient drought tolerant over Zarfam variety. Results show that with increasing stress severity in all growing stages, the decrease in RWC and increase in RS and T_c was higher when compared with the control which asserts their susceptibility to soil water condition and leaf water potential. Potassium application, in both stressed/non-stressed conditions caused a lower RS and T_c, but increased RWC. To sum up, K_2SO_4 could ameliorate negative effects of water stress on physiological properties and consequently improve them. RWC, RS, T_c and ΔT, are beneficial indices for screening large numbers of drought-tolerant rapeseed varieties in a short time at critical stages of crop growth.

Key words: *Brassica napus* L., canopy temperature, potassium fertilizer, relative water content, stomatal resistance, water deficit stress.

INTRODUCTION

Drought is one the most important abiotic stresses which causes serious damages in plants and it is recognized as a limiting factor for growth and production (Farooq et al., 2009; Yarnia et al., 2011). To meet the food requirement needs of the growing population, drought tolerant plants should be increased (Mahajan and Tuteja, 2005). The drought stress has an influence on physiological characteristics such as relative water content (Fanaei et al., 2009), stomatal conductance (Kauser et al., 2006), leaf temperature (Pasban Eslam et al., 2000), leaf water and osmotic potential (Chhabra et al., 2007). Stomatal control of water loss has been identified as an early event in plant response to water deficit under field conditions leading to limitation of carbon uptake by the leaves (Cornic and Massacci, 1996). Stomata close in response either to a decline in leaf turgor and water potential, or to a low-humidity atmosphere (Maroco et al., 1997). As a rule, stomatal responses are more closely linked to soil moisture content than to leaf water status. This suggests

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that stomata responds to chemical signals (abscisic acid/ABA) produced by dehydrating roots (Davies and Zhang, 1991). Since stomata are regulating agent of CO_2 exchange and leaf water vapor diffusion, the initial decrease in photosynthesis under moisture stress condition is normally done by the alteration in CO_2 conductance (Emam and Niknejad, 2004). There are some evidences which prove that the stomatal closure is firstly related to the reduction in photosynthesis and CO_2 concentration inside the leaf and in the chloroplast in moisture-limited condition (Yordanov et al., 2003; Hopkins and Hüner, 2004). Pasban Eslam et al. (2000) reported that in moisture stress condition, there was a decrease in the stomatal conductance (SC), leaf water potential (LWP), leaf relative water content (LRWC) and crop temperature stability (CTS), and there was an increase in specific leaf weight (dry matter per unit leaf area– SLW) and leaf temperature (T_L) in all varieties of rapeseed. However, varieties with the highest osmotic adjustment ability under stress appeared to have the least decrease in stomatal conductance, water potential, RWC and CTS. These researchers introduced the crop temperature stability as a more accurate index for the leaf temperature and water potential to drought. Kumar and Singh (1998) reported the positive correlation with the leaf water vapor diffusion and a negative correlation with the total amount of water lost through the leaves for the seed yield in *Brassica* species. They claimed that varieties with higher degree of osmotic adjustment would get cooler through transpiration, leaf diffusion and stomatal conductance; therefore, through the continual soil water absorption in water deficit condition especially in reproductive stages, they are largely able to preserve the leaf gas exchange. In other words, in these varieties, the root expansion continued during the reproductive stages. In a way, the continuity of gas exchange and leaf photosynthesis results in allocation of required photosynthetic material to the root parts and thereafter the crops growth and development. Kumar and Singh (1998) introduced high amounts of leaf relative water content as one of the characteristics which may influence the continual growth of rapeseed under drought stress. They concluded that with increased water deficit, the rapeseed varieties having high osmotic adjacent which are facing a sharp drop in leaf cellular osmotic potential can permit maintenance of a higher turgor potential (TP) and RWC.

In recent decade, much effort has been done to determine the relationship between crop temperature and water stress. Canopy temperature (T_c) is often a valid indicator of crop water stress (Moaveni and Changizi, 2007). Canopy measurements in stress condition have been reported as an acceptable criterion in determining the drought stress in wheat (*Triticum aestivum* L.) by Lotf Ali Ayeneh (2006), soybean (*Glycine max*) by Azizi (2000), cotton (*Gossypium hirsutum* L.) by Pervez et al. (2004) and rapeseed (*Brassica napus* L.) by Fanaei et al. (2009). Golhar and Dhopte (1996), with the hybridization plans under drought condition, reported lower leaf temperature and higher stomatal density on the upper leaf surface in mustard. Other researches also reported close associations between osmotic adjustment and both stomatal conductance and canopy temperature in wheat (Morgan et al., 1986), sorghum (Jones and Turner, 1978), cotton (Brown et al., 1976) and many *Brassica* species (Kumar et al., 1984). Kumar and Singh (1998) have investigated the canopy-air temperature difference in 22 genotypes of five *Brassica* species on two different maturity groups during the stage of pod formation and under soil moisture stress. As compared to rapeseed (*B. napus*) and field mustard (*Brassica rapa*), the absolute magnitude of cooling through transpiration in mid-day in Indian mustard (*Brassica juncea*) and Ethiopian mustard (*Brassica carinata*) was higher. The range of genetic diversity for the early-season genotypes and late-season genotypes was ½ to 4°C and ½ to 5°C, respectively. There was a positive and significant correlation between the amount of ΔT ($T_c - T_a$) in *Brassica* types in mid-day, and stomatal conductance, plant osmoregulation and seed yield.

Potassium increases the plant's drought resistance through its functions in stomatal regulation, osmo-regulation, energy status, charge balance, protein synthesis and homeostasis (Marschner, 1995). Elumalai et al. (2002) reported that potassium has a crucial role in stomatal conductance, osmoregulation, enzymatic activity, cellular expansion, ions neutralization and membrane polarization. In potassium deficiency conditions, the plants become more susceptible to the environmental stresses; therefore, as a result of stress condition, the release of active oxygen radicals in plants is highly stimulated (Cakmak, 2005). Mohammad and Naseem (2006) attributed the increasing effect of potassium application on photosynthesis to its functional role in stomatal activity for exchanging water vapor, carbon dioxide and oxygen and also its direct/indirect effect on enzymatic activity. They have declared that the potassium, by increasing the nitrate reductase activity, leads to efficient formation of molecules with nitrogen in their structure, which is responsible for synthesis of proteins and enzymes. These molecules are necessary for photosynthesis, ATP production, speed control of photosynthesis and sugar transmission via phloem vessels to other parts of the plant for application and conservation of ATP among other process. Egilla et al. (2005) showed that the leaf water content (LWC) and leaf water relations improved by lowering the osmotic potential when plants with potassium deficiency were compared with plants having sufficient potassium. Indeed, the high potassium consumption in both stressed and non-stressed conditions stabilized the net rate of photosynthesis, transpiration and stomatal conductance. In plants with potassium deficiency, the leaf water content, turgor potential, net photosynthesis, transpiration, stomatal conductance and water use efficiency were consistently lower as compared to the plants having sufficient potassium. Sharma et al. (1992) observed that among different *Brassica* types, rapeseed decreased the most in photosynthesis speed and relative water content, and it responded better in potassium application. Umar (2006) reported that potassium absorption increased the relative water content, nitrate reductase activity and seed yield through using 60 mg potassium/kg soil in non-stressed condition. However, under drought stress condition, the increase in the above mentioned traits was observed through using 90 mg potassium/kg soil. In other words, under drought stress condition, the need for potassium increases. Damon et al. (2007) also reported the inter-species differences, considering the potassium uptake efficiency in rapeseed. In plants subjected to drought stress, the accumulation of K^+ may be more important than the production of organic solutes during the initial adjustment phase, because osmotic adjustment through ion uptake like K^+ is more energy efficient (Hsiao, 1973). Fusheng (2006) has revealed that lower water loss of plants well supplied with K^+ is due to a reduction in transpiration which not only depends on the osmotic potential of mesophyll cells but also is controlled to a large extent by opening and closing of stomata. The effect of potassium fertilizer as an ameliorator agent of physiological indices under drought stress condition has been evaluated in an experiment conducted by Fanaei et al. (2009) on *Brassica* species. They found that stomatal conductance, chlorophyll content (value) and leaf temperature are suitable indices for screening drought tolerant varieties.

Keeping in view the importance of the studied factors, the present study was done to investigate the effect of different irrigation regimes and potassium levels on physiological characteristics of two rapeseed varieties.

MATERIALS AND METHODS

Site description and soil type

This research was undertaken in an experimental area, Qazvin region, Iran (latitude 36° 18´ N, longitude 49° 57´ E) during 2008–2009 and 2009–2010 growing seasons. This region has a semi-arid climate (312 mm annual rainfall). The soil of the experimental site was a clay loam, potassium content of 135 mg kg^{-1} with a pH of 7.8 and EC = 1.33 dS/m.

Experimental design and treatments

The experiment was factorial based on a randomized complete block design (RCBD) with three replications. The treatments were combination of 3 irrigation regimes (I_1–irrigation after 40%, I_2–irrigation after 60% and I_3– irrigation after 80% soil water depletion) and three potassium levels (K_1– 0, K_2– 100 and K_3– 200 kg K_2SO_4/ha) as well as two variety of rapeseed including “Zarfam” and “Opera”. The potassium levels were recommended by seed and plant improvement institute (SPII), Oilseed Crops Research Department of Karaj, Iran (based on the soil test results). The phenological characteristics of studied varieties are shown in Figure 1. Water deficit stress was based on irrigation, scheduled in terms of different percentages of soil water depletion (SWD) (Alizadeh, 2005). Combined treatments were randomized in the experimental units.

Management strategy

In both cropping seasons (2008–2009 and 2009–2010), the sowing was done manually in a wet planting pattern. The planting dates in the first and second year were on 29 October. Each experimental plot included 6 planting rows with 4 m length, 30 cm within the rows and in-row spacing of 4 cm. The net plot area size was 7.2 m^2. According to the soil test results, 180 kg N/ha from source of urea in 30, 40 and 30% splits were applied to the soil before sowing (A), at the end of rosette stage (B) and at the beginning of flowering stage (C). Various rates of potassium from K_2SO_4 were distributed in the studied plots after the final land leveling and before the sowing. In 4-6 true leaf stage, hand-weeding was performed together with the thinning operation. Eradicating aphids during the flowering and siliqua formation stage was done by using Endosulfan (an off-patent organochlorine insecticide) by the rate of 2 ml/L. The latest method of Canola Council of Canada was used to explain the growth and development stages (Shirani Rad, 2002). The stress was included from sowing date to maturity. The re-irrigation was based on moisture depletion in effective root depth of about 40, 60 and 80% of the plant available water. The irrigation time of the plot was determined through the soil moisture curve (*pF*) and by using TRIM-TDR (time domain reflectometry) soil moisture meter. Thus, the pipes, which are designed for TDR system, were installed at the depth of one meter between the rows at the time of plants’ 2 to 4 leaf stage. Then, the water depletion of experimental unit was observed at interval of every 3 days. The volume of water applied to each unit at each time of irrigation was calculated using the following equation (Alizadeh, 2005):

$$V_m = (Fc - \theta) \times BD \times A \times D / ea \quad (1)$$

Where, V_m is the volume of irrigation water based on cubic meter; *F*c is the soil moisture weight percentage at field capacity; θ is the soil moisture percentage with treatments; *BD* is the apparent specific weight of soil (g/m^2); *A* is the main plot’s area (m^2); *D* is the effective root depth (m) at the time of drought stress and ‘*ea’* is the irrigation water usage (95%). After estimating the amount of required water for the treatments by the water tank indicator, water entered the plots precisely and in a controlled manner. The number of irrigation for the treatments with 40, 60 and 80% of soil moisture depletion during the first and second year of study was 2, 3 and 4 times, and 1, 3 and 4 times, respectively. Crop management practices were operated as reguired during the growing season.

Estimation of physiological indices

Sampling

Measurements of physiological traits were done in 3 turns at a particular time of the day from 11.00 to 13.00 h at vegetative (VS), 50% flowering (50% F) and 100% siliqua formation (FS) stages prior to irrigation. The following parameters were determined: Leaf relative water content (RWC), leaf stomatal resistance (RS), canopy temperature (T_c) and the difference between canopy and air temperature (ΔT). The youngest fully expanded leaf (third from the apex), intact and full sunlit leaves per plot was used for various measurements.

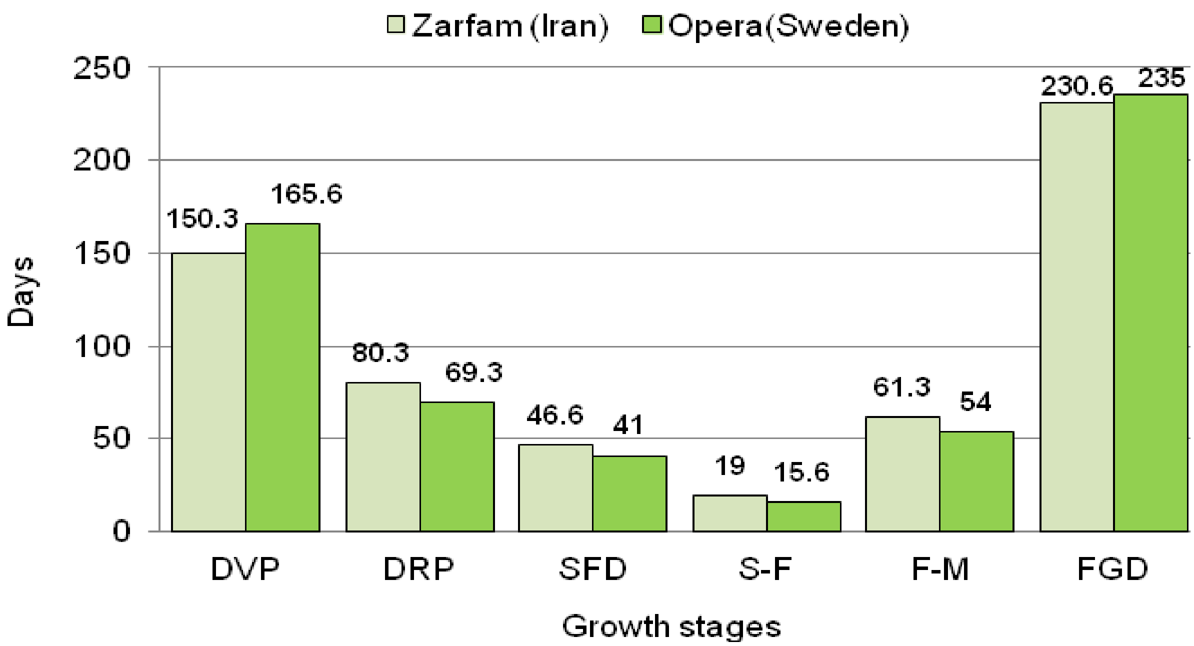

Figure 1. Comparison of different phenological stages in winter rapeseed varieties (Zarfam and Opera).The growth stages includie: DVP, the duration of the vegetative phase; DRP, the duration of the reproductive phase; SFD, seed-filling duration; S-F, Stem elongation-flowering stage; F-P, flowering stage-physiological maturity; FGD, full growth duration. The data was gotten from the record of Oilseed Crops Research Department, Seed and Plant Improvement Institute, Karaj, Iran.

Determination of leaf relative water content (RWC)

Leaf RWC is defined as the ratio of the water volume in a leaf to the maximum water volume in the same leaf at full turgor. Full turgor can be controlled by the crop in response to water stress. RWC was described as follows:

$$RWC(\%) = \left[\frac{(FW - DW)}{(TW - DW)}\right] \times 100 \quad (2)$$

Where, *FW* is the fresh weight; *DW* is the dry weight and *TW* is the turgid weight (Lazcano-Ferrat and Lovatt, 1999).

For RWC determination, leaf disks were taken from three to four leaves (5 circle disks, 1 cm^2) of similar physiological maturity and weighed (FW); then, the samples were immediately hydrated to full turgidity for 4 h under normal room light (dim light) and temperature. Afterwards, turgid leaf disks weights were measured (TW). Samples were dried in oven at 80°C for 24 h and weighted (DW).

Measurement of leaf stomatal resistance (RS)

Leaf stomatal resistance (Autoporometer, AP4, Delta-T Devices Ltd, Cambridge, UK) was measured as an average of ten leaves and expressed as second per centimeter (s cm^{-1}) (Pasban Eslam et al., 2000).

Measurements of canopy temperature (T_c) and ΔT

Canopy temperature (T_c), was measured by a portable infrared thermometer (0.99 EM, USA) and ambient air temperature (T_a) was measured using a hydrargyric thermometer. The difference between canopy and air temperature was recorded as ΔT (Azizi, 2000; Kluitenberg and Biggar, 1992). Initially, four random point selected per plot and T_c values were noted, then their averages inscripted mean canopy temperature. Measurements were done from flowering stage until maturity prior to irrigation (Azizi, 2000).

$$\Delta T = T_c - T_a \quad (3)$$

Where, T_c and T_a are canopy and ambient air temperature, respectively.

Statistical analysis

Data were subjected to analysis of variance (ANOVA), using the MSTATC statistical package (MSTAT-C, Version1.41, Crop and Sciences Department, Michigan State University, USA), and Duncan's multiple range test (at the 0.05 probability level) was employed for the mean comparisons.

RESULTS AND DISCUSSION

Relative water content (RWC)

RWC is the appropriate trait of plant water status pertaining to the physiological consequence of cellular water deficit. As shown in Table 1, the individual effect of irrigation, potassium sulphate (K_2SO_4) in different sampling stages on RWC were highly significant (P<0.01). In this study, RWC was drastically affected by the years of

Table 1. The mean squares of ANOVA for physiological characteristics of RWC, RS and T_c in different growing stages via combined analysis of 2008–2009 and 2009–2010 data.

Source of Variance	df	VG		50% F			FS		
		RWC	RS	RWC	RS	T_c	RWC	RS	T_c
Y	1	172.162**	55.176**	1142.162**	21.022*	1.015^{ns}	4.015^{ns}	13.021^{ns}	889.324**
E_a	4	8.022	0.141	36.127	2.025	4.635	15.105	4.027	5.325
V	1	76.121**	0.421^{ns}	14.121^{ns}	2.003**	1.486^{ns}	536.025**	17.023**	19.337**
Y V	1	12.025*	3.012*	178.022**	0.023^{ns}	2.623*	227.016**	2.025ns	1.018^{ns}
I	2	1843.026**	159.117**	2598.083**	254.027**	49.121**	1291.136**	196.175**	116.438**
Y I	2	197.075**	22.136**	44.136**	2.025**	17.017**	72.068**	5.022**	11.079**
V I	2	5.126^{ns}	2.033*	20.175*	3.026**	7.025**	50.128**	3.026*	1.046^{ns}
Y V I	2	30.137**	2.011*	23.135*	0.612^{ns}	5.012**	5.021^{ns}	2.497^{ns}	2.026**
K	2	232.161**	7.022**	416.027**	18.126**	41.129**	481.162**	23.162**	34.038**
Y K	2	7.126^{ns}	0.248^{ns}	10.112^{ns}	2.025**	0.935^{ns}	4.027^{ns}	1.785^{ns}	0.486^{ns}
V K	2	1.352^{ns}	0.008^{ns}	1.248^{ns}	0.563^{ns}	0.009^{ns}	4.063^{ns}	0.416^{ns}	0.237^{ns}
Y V K	2	0.036^{ns}	0.563^{ns}	7.182^{ns}	0.628^{ns}	0.739^{ns}	12.128^{ns}	1.286^{ns}	0.622^{ns}
I K	4	19.021^{ns}	1.462*	7.023^{ns}	1.621**	0.442^{ns}	4.028^{ns}	2.128*	0.896^{ns}
Y I K	4	2.023^{ns}	0.267^{ns}	3.112^{ns}	0.928**	0.386^{ns}	3.027^{ns}	1.447^{ns}	0.567^{ns}
V I K	4	9.026*	0.078^{ns}	5.026^{ns}	0.238^{ns}	0.423^{ns}	6.025^{ns}	0.348^{ns}	0.429^{ns}
Y V I K	4	4.027^{ns}	0.463^{ns}	2.175^{ns}	0.069^{ns}	0.551^{ns}	2.027^{ns}	0.487^{ns}	0.098^{ns}
E	68	3.037	0.459	6.023	0.262	0.538	4.025	0.822	0.418
C.V (%)	–	2.76	12.38	3.76	10.12	3.23	3.14	14.16	3.12

*****– $P < 0.05$, ******– $P < 0.01$, ns– $P > 0.05$; Y– year effect, V– variety effect, I– irrigation effect, K– potassium sulphate effect. Y V, Y I, V I, Y V I, Y K, V K, Y V K, I K, Y I K, V I K and Y V I K represent interaction terms between the treatment factors.VG– vegetative growing stage, 50% F– 50% flowering stage, FS– 100% siliqua formation stage. RWC, leaf relative water content; RS, leaf stomatal resistance; T_c, canopy temperature; VG, vegetative growing stage; 50% F, 50% flowering stage; FS, 100% siliqua formation stage.

study during the vegetative growth (VG) and 50% of flowering stages (50% F). The highest and lowest RWC was observed in the second year of study during the vegetative growth and 100% siliqua formation stages (FS), respectively (Table 2).

During VG and FS stages, the two tested varieties showed a significant difference in terms of the said trait; whereas, during the VG stage, Zarfam variety had the highest RWC as compared to Opera variety. However, the highest RWC was observed in Opera variety during the FS stage (Table 2). Kage et al. (2004) attributed the difference in the leaf relative water content of rapeseed varieties to their root system variations. The variety differences were also reported in the pattern of considering the soil moisture and root depth in Indian mustard (Kumar and Singh, 1998). With the increase in stress intensity in all the phenological stages, the decline in RWC was higher as compared to the control treatment (Table 2); accordingly, the lowest RWC was observed in the treatment I_3 (irrigation after 80% soil water depletion). As compared to VG stage, the decrease in RWC in the plots with 40, 60 and 80% soil water depletion (SWD) in FS stage was 16, 17 and 21% respectively. The increase in stress duration and intensity under severe stress treatment (irrigation after 80% SWD) as compared to the control treatment (irrigation after 40% SWD) and moderate stress (irrigation after 60% SWD), can justify the said reduction. The increase in RWC by drought stress was also reported by other workers (Sharma et al., 1992; Egilla et al., 2005; Pasban Eslam, 2009). The variations in RWC during different growing stages signify the high sensitivity of this trait to the changes in soil water condition and leaf water potential.

The study on the trend of RWC changes show that with increase in plant age, a decrease was observed in RWC under the irrigation regimes in different sampling stages (Table 2). Palomo et al. (1999) reported the increase in RWC at the beginning of the season and a decrease in later stages. Their results were in line with our findings. The RWC variations influenced by potassium levels in different sampling stages are shown in Table 2. The increase in K_2SO_4 application improved RWC in all growing stages in such a way that the increase was observed in VG stage from 82% in K_1 (non-potassium application treatment) to 87% by applying 200 kg/ha of K_2SO_4, in 50% F stage, from 77 to 84%, and in FS stage from 68 to 75%. This suggests that potassium has a positive role in turgidity maintenance and continual cell growth (Egilla et al., 2005; Fusheng, 2006).

The interaction effect of variety and irrigation on RWC was significant in 50% F (P<0.05) and FS (P<0.01) stages. In VG stage, Zarfam had the highest RWC during the various moisture conditions as compared to Opera. Yet, in FS and 50% F stages, the Opera was better

Table 2. Individual effects of year, variety, irrigation and potassium sulphate levels on physiological characteristics of RWC, RS, T_c and *Δt (T_c-T_a) in vegetative, 50% flowering and 100% siliqua formation stages of rapeseed.

Treatment	VG		50% F				FS			
	RWC (%)	RS (s cm $^{-1}$)	RWC (%)	RS (s cm $^{-1}$)	T_c (°C)	ΔT (°C)	RWC (%)	RS (s cm $^{-1}$)	T_c (°C)	ΔT (°C)
Y_1	83.44^b	4.41^a	77.09^b	5.15^a	26.18^a	---	71.72^a	6.33^a	32.09^a	---
Y_2	85.98^a	2.88^b	83.66^a	4.25^b	25.99^a	---	71.73^a	7.01^a	26.31^b	---
V_1	85.55^a	3.66^a	80.02^a	4.82^a	25.98^a	-3.31^a	64.27^b	7.06^a	29.61^a	-3.25^a
V_2	83.66^b	3.54^a	80.74^a	4.59^b	25.19^a	-3.09^a	73.81^a	6.27^b	28.79^b	-4.04^b
I_1	90.83^a	1.89^c	89.06^a	1.91^c	25.25^a	-4.44^c	77.68^a	4.26^c	27.32^c	-5.48^c
I_2	86.55^b	2.94^b	80.15^b	4.97^b	26.19^a	-3.02^b	71.41^b	6.78^b	29.34^b	-3.74^b
I_3	76.74^c	5.96^a	71.91^c	7.24^a	27.22^a	-2.14^a	65.59^c	8.97^a	30.94^a	-1.98^a
K_1	82.24^c	4.05^a	76.89^c	5.41^a	27.23^a	-2.05^a	67.73^c	7.55^a	30.25^a	-2.65^a
K_2	84.52^b	3.56^b	80.51^b	4.72^b	25.93^b	-3.35^b	71.86^b	6.47^b	29.03^b	-3.79^b
K_3	87.35^a	3.18^c	83.75^a	3.99^c	25.11^c	-4.21^c	75.09^a	5.99^c	28.33^c	-4.51^c

Means not sharing a common letter in a column differ significantly at 0.05% level of probability using Duncan multiple rang test (DMRT). Y– Cropping year (Y_1: first year; Y_2: second year), V– Variety (V_1: Zarfam; V_2: Opera), I– Irrigation (I_1: irrigation after 40%, I_2: irrigation after 60% and I_3: irrigation after 80% soil water depletion), K– potassium sulphate (K_1:0 as control, K_2:100 and K_3: 200 kg/ha K_2SO_4). *Δt– the difference between canopy (T_c) and ambient air temperature (Ta). RWC, leaf relative water content; RS, leaf stomatal resistance; T_c, canopy temperature; ΔT, the difference between canopy and air temperature; VG, vegetative growing stage; 50% F, 50% flowering stage; FS, 100% siliqua formation stage.

(Table 3). The result indicates the superiority of Opera in mid and late growing season to drought stress tolerance. Ma et al. (2006) reported a different reaction through osmoregulation in rapeseed and mustard at different growth stages and showed that in the vegetative stages, they showed osmoregulation under the drought stress; however, in flowering stage, only mustard showed osmoregulation. Wright et al. (1996) reported a positive correlation between the osmoregulation and leaf RWC of rapeseed and Indian mustard.

Interaction effects of irrigation regime and potassium on RWC was not significant at VG, 50% F and FS stages (P>0.05, Table 1). The highest RWC was observed in favorable and unfavorable moisture conditions with the highest amount of applied potassium (200 kg K_2SO_4/ha); thus, in desirable moisture conditions, the highest RWC was observed in 100 and 200 kg K_2SO_4/ha which had no significant difference. However, in the limited moisture condition, the consumption of 200 kg K_2SO_4/ha indicated considerable difference as compared to the lower amounts. Potassium intake has increased RWC in favorable moisture conditions and in drought stress condition from 2 to 5% and 8 to 10%, respectively (Table 3). The high drought stress intensity increases the potassium requirement for improving the water status and maintaining photosynthesis (Umar, 2006). Apart from the disorder in photosynthesis electron transport chain, the production of active oxygen formed by potassium deficiency is increased by NADPH oxidation (an important source for active oxygen production in plants subjected to potassium deficiency stress). It should be noted that the plants become more susceptible to environmental stress in potassium deficiency condition (Cakmak, 2005).

Stomatal resistance (RS)

Results of combined analysis of variance indicated that stomatal resistant (RS) in different growing stages (sampling stages) was significantly affected by irrigation, potassium and interaction thereof as well as V×I interaction (Table 1). The RS in VG and 50% F stages was affected by the years of study and the maximum RS was observed in VG and 50% F stages during the first year and in FS stage during the second year. The mean comparison of RS at different growing stages showed its increasing trend by increase in age of the plant (Table 2). The varieties regarding RS showed a significant difference in 50% F and FS stages; hence, Zarfam with mean of 4.82 and 7.06 (s cm^{-1}) had the highest RS as compared to Opera with mean of 4.59 and 6.27 (s cm^{-1}) during the stages of 50% F and FS (Table 2). Pasban Eslam et al. (2000) and Sadaqat et al. (2003) reported the existence of genetic differences for stomatal resistance in rapeseed. In this experiment, among different growing stages, the highest RS was observed at the end of growing season. These results coincide with those obtained in a trial carried out by Naderikharaji et al. (2008). In all sampling stages, as compared to the control treatment, the RS was higher in I_2 and I_3 (irrigation after 60 and 80% SWD) treatments due to the increase in drought stress intensity (Table 2). The stomatal closure is the most significant factor which is associated with the cessation of photosynthesis at early stages of water deficiency (Yordanov et al., 2003). The negative effect of water deficit stress on RS was observed in studies of Fanaei et al. (2009) and Pasban Eslam et al. (2000). The highest RS in all growth stages was achieved by non-potassium application; and by increase in potassium

Table 3: Interaction effect of variety and irrigation (V×I) as well as irrigation and potassium (I×K) on physiological characteristics of RWC (relative water content), RS (stomatal resistance), T_c (canopy temperature) and Δt (T_c-T_a) in different growing stages.

Interactions	VG		50% F				FS			
	RWC (%)	RS (s cm^{-1})	RWC (%)	RS (s cm^{-1})	T_c (°C)	Δt (°C)	RWC (%)	RS (s cm^{-1})	T_c (°C)	Δt (°C)
Variety × Irrigation										
V_1I_1	91.26a	1.83d	89.47a	1.87d	24.31e	-4.98e	76.48b	4.37e	27.82a	-5.01a
V_1I_2	87.51a	2.89c	79.77b	4.95c	26.14c	-3.14c	69.41d	7.16c	29.57a	-3.24a
V_1I_3	77.89a	6.24a	70.81d	7.64a	27.48a	-1.81a	62.05e	9.65a	31.46a	-1.52a
V_2I_1	90.41a	1.95d	88.66a	1.93d	25.37d	-3.91d	78.89a	4.14e	26.84a	-5.98a
V_2I_2	85.61a	2.98c	80.55b	4.99c	26.43bc	-2.89bc	73.41c	6.38d	29.12a	-3.71a
V_2I_3	75.59a	5.69b	73.01c	6.84b	26.81b	-2.48b	69.12d	8.28b	30.41a	-2.45a
Irrigation × potassium										
I_1K_1	89.55a	2.05f	86.22a	2.29g	25.82a	-3.46a	74.24a	4.71e	28.08a	-4.74a
I_1K_2	90.82a	1.92f	89.22a	1.83h	24.71a	-4.57a	78.36a	4.11e	27.26a	-5.56a
I_1K_3	92.13a	1.69f	91.75a	1.57h	23.97a	-5.31a	80.46a	3.98e	26.65a	-6.17a
I_2K_1	84.09a	3.34d	75.85a	5.94d	27.45a	-1.83a	67.42a	7.66c	30.51a	-2.31a
I_2K_2	86.18a	2.78e	80.61a	5.09e	26.18a	-3.11a	71.54a	6.67d	29.22a	-3.61a
I_2K_3	89.36a	2.68e	84.01a	3.89f	25.22a	-4.12a	75.28a	6.01d	28.32a	-4.51a
I_3K_1	73.08a	6.74a	68.61a	7.97a	28.41a	-1.88a	61.54a	10.28a	32.17a	-1.91a
I_3K_2	76.54a	5.96b	71.66a	7.22b	26.91a	-2.38a	65.68a	8.65b	30.61a	-2.19a
I_3K_3	80.59a	5.19c	75.48a	6.52c	26.12a	-3.16a	69.55a	7.98bc	30.84a	-2.86a

Note. Means not sharing a common letter in a column differ significantly at 0.05% level of probability. VG–vegetative growing stage, 50% F– 50% flowering stage, FS– 100% siliqua formation stage.

application, the RS was decreased. By a careful consideration of the decreasing trend of RS at different sampling stages, it is clear that the RS drop rate caused by potassium application in VG stage (0.87 s cm^{-1}) was lower as compared to 50% F (1.42 s cm^{-1}) and FS (1.56 s cm^{-1}) stages (Table 2). With increase in plant age and under more severe condition of moisture deficit, the role of potassium become more evident and its application have more positive effect in increasing the stomatal conductance. The increase in the stomatal conductance in both varieties, which is as a result of the increased availability of potassium in growing environment, is in accordance with the results reported by Sharma et al. (1992) and Pervez et al. (2004). Many researchers pointed out the crucial role of potassium in stomatal conductance. They attributed the increasing effect of potassium application on photosynthesis to its effect on stomatal activity for exchange of carbon dioxide (CO_2), water vapor and oxygen (Mohammad and Naseem, 2006; Egilla et al., 2005; Pervez et al., 2004). Potassium deficiency causes less transpiration in the leaves and this decrease is not irrelevant to the increase in stomatal resistance and less water conductance for the roots. The mechanism of stomatal opening and closing is related to status of potassium diffusion in the guard cells. Thus, in plants with potassium deficiency, a varying response and stomatal movement can be observed. The potassium supply for stomatal opening might be in the leaf epidermal cells, mesophyll cells or both of them (Leigh, 2001).

In VG, 50% F and FS stages, the stomatal resistance was affected by the interaction of variety and irrigation which resulted to lower RS in Zarfam during the VG and 50% F stages in the mild drought stress condition (I_2). However, Opera had lower RS in FS stage. As compared to the favorable moisture and mild stress, the severe drought stress enhanced the RS in both varieties (Table 3). However, in severe drought stress condition of all growth stages, Opera had lower RS as compared to Zarfam. The superior root system and the ability to make water available from deeper soil layers for turgidity maintenance and higher osmoregulation were introduced as characteristics of drought tolerant varieties in *Brassica* species (Kumar and Singh, 1998). In Zarfam, the stomatal conductance has a close relationship with leaf RWC and turgor pressure, under drought condition; hence, the decrease in leaf RWC under the water deficit condition causes the decrease in stomatal conductance (stomatal closure) and CO_2 diffusion into the leaves which ultimately decreases the photosynthetic activity (Pasban Eslam et al., 2000). Means comparison of interaction effects revealed that with the increase in applied potassium (from 0 to 200 kg K_2SO_4/ha) at all sampling stages (VG, 50% F and FS) for every irrigation pattern, a significant reduction in RS was observed (Table 3). Potassium application imposed a positive effect on RS in all studied drought conditions, but its influence under severe water stress (I_3) was outstanding. The significant decrease in RS in response to potassium application under stressed/non-stressed conditions might be due to improved turgor and stomatal opening. Accumulation and release of potassium by stomatal guard cells lead to changes in their turgor, resulting in stomatal opening and closing (Fischer and Hsiao, 1968). Potassium increases the plant's drought resistance through its functions in stomatal regulation. Therefore, for plants growing in drought conditions, accumulating abundant K^+ in their tissues may play an important role in water uptake along a soil-plant gradient (Fanaei et al., 2009). Numerous studies have shown that the application of K fertilizer mitigates the adverse effects of drought on plant growth (Andersen et al., 1992; Tiwari et al., 1998; Sangakkara et al., 2001; Egilla et al., 2005; Singh and Kuhad, 2005; Fanaei et al., 2009) which confirmed our findings.

Canopy temperature (T_c) and ΔT (T_c-T_a)

Leaf and canopy temperature measurement is a prevalent method for assessing the drought stress intensity in crops (Kumar and Singh, 1998; Pasban Eslam, 2009; Fanaei et al., 2009). Making use of the canopy temperature (T_c) for identifying the crop water status and water requirement is based on the premise that the transpiration is remarkably effective in cooling the leaves. With the limited access to water, the transpiration will decrease and subsequently increase the leaf temperature (T_L) due to the continual absorption of radiation. The difference between the canopy and air temperature (ΔT) can be used as a guide for irrigation scheduling (Azizi, 2000; Kumar and Singh, 1998).

It is also evident from the data (Table 1) that T_c was only influenced by the years of study and tested varieties (Zarfam, Opera) in the FS phase of growth. While interaction effect of variety and irrigation in stage of 50% F affected this trait. In addition, individual effect of irrigation and potassium in both stages of 50% F and FS were significantly affected by T_c, whereas T_c was not affected by their interaction. The leaf temperature (T_L) had an increasing trend from the beginning of measurement to the end of the growing season. The highest T_c was obtained in the first year of study during the stages of 50% F and FS. Apparently, in the stages of 50% F and FS, the increase rate of T_c in the first year as compared to the second year was 0.19 and 5.78°C, respectively (Table 2). The obtained results showed non-significant difference among the tested varieties in terms of T_c and ΔT; however, the difference was significant in FS stage (Table 2). Accordingly, the two varieties reacted differently when exposed to drought condition, and Opera in FS stage had higher cooling rate in the leaves than Zarfam. Similar results were obtained in the experiments carried out by Kumar and Singh (1998) concerning the difference between canopy and air temperature in Indian mustard, Ethiopian mustard and rapeseed.

Pervez et al. (2004) also reported the differences of T_c in the range of 29.2 to 31.5°C in the different treatment. The mean comparison result also suggested a significant difference in T_c and ΔT in the irrigation regimes (Table 2). By increasing the drought stress intensity, the increase amount of T_c showed a difference as compared to the control treatment (I_1). Whereas, an increase in temperature was accompanied by an increase in plant age at the next stage of growth in plots influenced by I_1, I_2 and I_3 (irrigation after 40, 60 and 80% SWD), treatments in FS stage were 2.7, 3.15 and 3.72°C, respectively as compared to the stage of 50% F. The mean comparison according to the ΔT showed that in I_3 treatment (irrigation after 80% SWD) due to the long-term stress, the value of ΔT became smaller (Table 2). It was observed earlier that RS showed an obvious difference in this treatment as compared to the control treatment. Thus, this is the reason why plant prevents water loss via transpiration by closing its open stomata. In contrast, the leaf temperature in canopies (plots) which was affected by this treatment increased to the extent that its absolute difference with the air temperature decreased. This outcome was reported for the soybean by Azizi (2000) as well as the rapeseed and mustard by Pasban Eslam (2009), Kumar and Singh (1998) and Fanaei et al. (2009). The canopy temperature in both varieties was increased by increasing the restriction on water availability, but the increasing trend of T_c in Zarfam was higher as compared to Opera (Table 3). Probably, higher RWC and stomatal conductance in Opera were the reasons for its superiority. A very thin cuticle and thickness of the leaves are also effective in creating the temperature differences between the varieties. The thin cuticle of the leaf causes the efflux of water vapor from the leaves into the ambient air and results in plant cooling (Fanaei et al., 2009). Pasban Eslam et al. (2000) reported the increase in T_c in all *Brassica* varieties under moisture stress condition which is in conformity with the results of this study. Irrespective of the growing stages, the rise in soil potassium amount caused adjustment and drop in temperature as compared to the control (I_1) treatment (Table 2). As comparing to 50% F stage, potassium sulphate application in the rate of 100 and 200 kg/ha has decreased T_c in FS stage to 3.1 and 3.22°C, respectively. In this respect, similar result was achieved by Pervez et al. (2004) on cotton (*Gossypium hirsutum* L.). The high amount of potassium application increased the absolute value of ΔT (Table 2) which means that the crop by balancing its stomatal movements and utilizing the mechanisms such as osmotic regulation can continue transpiration without any severe damage in photosynthesis process and become cool (Azizi , 2000).

Conclusion

It can be concluded that the two studied varieties had significant differences regarding some physiological properties during the different growing stages. Higher RWC and stomatal conductance (lower stomatal resistance) in Zarfam variety under suitable moisture condition and inversely higher RWC and stomatal conductance and lower canopy temperature in Opera variety under drought stress condition revealed that Opera performed better in limited moisture condition as compared to Zarfam. Although, the production potential of Opera is lower than that of Zarfam, due to this characteristic, Opera is more compatible with the arid areas. With increase in the drought stress intensity at all growing stages, the rate of decrease in RWC, the increase in RS and T_c was considerable as compared to the control treatment. The increase in soil potassium amount in both stressed/non-stressed conditions improved the physiological indices at all growth stages. Potassium sulphate (K_2SO_4) consumption increased RWC, decreased stomatal resistance and canopy temperature, and ultimately ameliorated the negative impact of drought stress. Based on the obtained result, it can be explained that RWC, RS, T_c and ΔT are beneficial indices for screening the drought tolerant rapeseed varieties.

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Abbreviations

***T_a*,** Ambient air temperature; ***T_c*,** canopy temperature; **RS,** leaf stomatal resistance; **RWC,** leaf relative water content; **ΔT,** the difference between canopy and air temperature; **VG,** vegetative growing stage; **50% F,** 50% flowering stage; **FS,** 100% siliqua formation stage; **SWD,** soil water depletion.

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