

The Influence of Drought Stress on Nutrients Uptake and Physiological Responses in Rapeseed (*Brassica napus* L.) Lines

Hassan Zali¹, Omid Sofalian¹, Tahereh Hasanloo^{2*},
Ali Asghari¹ and Mehrshad Zeinalabedini³

¹Plant Breeding of Department, University of Mohaghegh Ardabili, Ardabil, Iran.

²Department of Molecular Physiology,

Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.

³Department of Genomics, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.

(Received: 05 January 2015; accepted: 10 March 2015)

To study the effect of drought stress on nutrients uptake and physiological traits of seven rapeseed advanced lines, a two year experiment was conducted under greenhouse condition. The experiments were laid out in CRD factorial with three replications. A highly significant decline was observed in chlorophyll a and b content, relative water content, and biomass of oilseed rape lines under drought stress, while total soluble sugars content enhanced in this condition. The results showed that phosphate was the most abundant ion in leaves under both stress and non-stress conditions. The concentration of nutrients was also affected by drought and mostly diminished under stress condition. Water deficit significantly reduced nitrate, phosphate, sulfate, ammonium, calcium and manganese ions uptake, which was greater for phosphate than the other ions. In contrast, the concentration of nitrite increased. Also among inorganic forms of nitrogen (nitrate, nitrite and ammonium), the highest and the lowest sensitivity to soil moisture content was observed in ammonium and nitrate, respectively. In conclusion, improving nutritional status of rapeseed lines under drought stress condition is essential to diminish detrimental effects of water deficit.

Key words: Rapeseed (*Brassica napus*), Drought stress; Nutrient uptake; Physiological responses

Rapeseed (*Brassica napus* L.) is a major crop cultivated worldwide mainly for oil, human consumption and renewable fuel (Shiranirad *et al.*, 2014).

Large parts of the world are increasingly affected by drought. Drought stress is one of the most important abiotic factors which adversely affect growth, metabolism and yield of crops worldwide (Ghanbari *et al.*, 2013). Drought stress, during any particular growth stage of crops results in yield reduction. Under shortage of water, plants accumulate metabolites, such as sugars and

inorganic ion, to regulate osmotic potential (Zhang *et al.*, 2015).

Water deficit reduces the availability, uptake, translocation and metabolism of nutrients. A reduced transpiration rate because of water deficit diminishes the nutrient absorption and efficiency of their utilization (Rouphael *et al.*, 2012). Decreasing soil water under drought stress generally results in restricted total nutrient uptake and their decreased tissue concentrations in crop plants (Farooq *et al.*, 2009). A mementos effect of water deficit is on the acquisition of nutrients by the root and their transport to shoots. Lowered absorption of the inorganic nutrients can result from interference in nutrient uptake and the unloading mechanism, and reduced transpirational flow (Garg, 2003; McWilliams 2003). Also, plant

* To whom all correspondence should be addressed.
E-mail: Thasanloo@abrii.ac.ir

species and genotypes of a species may vary in their response to mineral uptake under drought stress (Farooq *et al.*, 2009).

Reduction of photosynthesis, enhance of root respiration leading to a diminution in the energy supply to the roots which restricted availability of energy for assimilation of some ions. For example, PO_3^{-4} and SO_2^{-4} and $\text{NO}_3^{-3}/\text{NH}_4^{+4}$ must be converted in energy-dependent processes before these ions can be used for plants growth (Grossman and Takahashi, 2001).

Inorganic forms of nitrogen are nitrate, nitrite, ammonium, and ammonia. Nitrate and ammonium are readily taken up by plants and beneficial for plant growth. Nitrite and ammonia are toxic to plants. Nitrogen is the mineral element that plants require in the largest amounts and is a constituent of many plant cell components, including amino and nucleic acids (Dijkstra *et al.*, 2015).

Ma *et al.* (2004) reported under drought stress condition, the accumulation of potassium may be more important than the production of organic solutes during the initial adjustment phase, because osmotic adjustment through ion uptake such as K is more energy efficient. Among nutrients, potassium ions help in osmotic adjustment (Farooq *et al.*, 2009). Potassium is essential for many physiological processes, such as protein synthesis, enzyme activation, cell expansion, photosynthesis, cell turgor and ion homeostasis in plant cells, energy status, and a competition of Na under saline conditions (Fournier *et al.*, 2005; Kanai *et al.*, 2007). Potassium increases the plant's drought tolerance through osmotic adjustment, maintains turgor pressure and reduces transpiration under drought conditions (Rouphael *et al.*, 2012).

Inorganic phosphorus is an essential macronutrient for plant growth and development. Phosphorus is a constituent of phosphoproteins, nucleic acids, adenosine triphosphate, phospholipids, and dinucleotides (Marschner, 2012). The positive effects of P on plant growth under drought have been attributed to an increase in the efficiency of water use, stomatal conductance, and photosynthesis, to higher cell membrane stability, and to effects on water relations (Rouphael *et al.*, 2012).

Chloride is a main osmotically active

solute in the vacuole involved in both turgor and osmoregulation processes, with implications for the suitable development of plants (Colmenero-Flores *et al.*, 2007). The sulfur is required for optimal growth of plants (Marschner, 2012). Sulfur is a key component in helping plant to cope with such abiotic stress (Chan *et al.*, 2013).

Calcium is a necessary plant nutrient and is required for structural roles in the cell wall and membranes, as a counteraction for inorganic and organic anions in the vacuole and as an intracellular messenger in the cytosol (Marschner, 2012). Moreover, it has an important role in response to drought stress (Dodd *et al.*, 2010).

Magnesium plays an essential role in photosynthesis, as it is the central atom in the chlorophyll molecule. It is involved in many enzyme reactions. It reacts with phosphorus in uptake and transport (Cakmak and Kirkby, 2008).

Several studies have shown the effects of drought stress on nutrients uptake in different crops (McWilliams, 2003; Pimratch *et al.*, 2013), but there is still insufficient data for effects of soil water deficit on anions and cations uptake in rapeseed cultivars. McWilliams (2003) pointed out that K uptake was hampered under drought stress in cotton. Peuke and Rennenberg (2004) indicated P and PO_3^{-4} uptake in the plant tissues reduced under water deficit stress.

Drought stress is a frequently abiotic stress which affects plant growth, development and anions and cations uptake. Ions play important roles in favorable responses of plants to water deficit. Therefore understanding water deficit impacts on nutrient uptake could be useful for improving plant tolerance under drought stress condition. Also, the data on the genotypic variation among rapeseed lines for nutrient uptakes across drought stress is still lacking. The aim of this study was to evaluate anions and cations uptake in rapeseed lines in response to drought stress.

MATERIALS AND METHODS

Plant materials and water deficit treatment

Two pot experiments were conducted at Agricultural Biotechnology Research Institute Of Iran (ABRII), Karaj, Iran, during 2013 - 2014. A factorial experiment arranged in completely randomized design (CRD) with three replications

was used. Analysis of variance (ANOVA) was conducted for two experiments and obtained mean values were compared using Duncan's Multiple Range Test. Seven rapeseed lines including L1, L2, L3, L4, L5, L6 and L7, provided by Seed and Plant Improvement Institute, Karaj, Iran, were used. Seeds were planted in plastic pots containing 3.5 kg mixed soil. The plants were grown in greenhouse under 13h d⁻¹ photoperiod and mean temperature of 25 ± 2 °C, for eight weeks. Thereafter, two irrigation levels, including well watering (WW; 90-100% soil water content [SWC]) and water withholding for 25 days (WD; 40-50% soil water content), were set as the experimental layout. After 25 days, relative water content and dry matter accumulation were measured.

Soil water content (SWC)

Soil water content (SWC) was calculated using the weight fraction as:

$$SWC (\%) = [(FW - DW)/DW] \times 100,$$

where FW is the fresh weight of a portion of the soil from the internal area of each pot and DW is the dry weight of the soil portion after drying in a hot air oven at 85 °C for 4 days.

Total sugar content analysis

Total sugar content was extracted from the dried leaves using the ethanol. Briefly, dried material (0.03 g) was homogenized in 1.5 ml of 80 % ethanol. The supernatant was collected after centrifuging at 5,000 rpm for 5 min. The removed supernatant placed in oven to evaporate the ethanol. After evaporation, the following chemicals were added: 10 ml distilled water, 470 µl 0.3 N Barium hydroxide, and 500 µl 5% zinc sulfate. The mixture was centrifuged at 10,000rpm for 10 min. The 0.5 ml supernatant was poured into a new tube and 0.5 ml phenol 5% and 2.5 ml sulfuric acid 98% were added. The samples were incubated at 25°C for 45 min. The sugar content was measured at 458 nm using a spectrophotometer (Walker *et al.*, 2008).

Chlorophyll a (chl-a), chlorophyll b (chl-b) and total chlorophyll (chl-t)

Chl-a, chl-b, and Chl-t were determined according to the method of Arnon (1949). Fresh leaves were taken from the plants and pulverized in 80% acetone. The absorbance of the extracts was measured at 663, 642 and 472 nm using a spectrophotometer.

Cation and anion analysis

Soluble cations were extracted from dried

leaves (0.05 g) by shaking for 4 h with 5 ml extraction solvent (0.1 N HCl) at 80 °C water bath. After cooling to room temperature for 24 h, the samples were then filtered and subjected to ion chromatography (IC; 850 Professional IC, Metrohm, Switzerland) with a Metrosep C2 250 column.

Dried leaf tissues were ground into powder (0.05 g) and were extracted with distilled water (10 ml) for 4 h at 80 °C water bath to determine the content of anions. The extract was filtered and subjected to the same IC but with a Metrosep Asupp7 column (Metrohm Company, 2011).

Statistical analysis

All calculations were carried out with Statistica, SPSS and Sigma plot 12.2 software. The normal distribution of the data was studied with the Shapiro-Wilk test at 95 %.

RESULTS AND DISCUSSION

Physiological indices

The effect of water stress on drought tolerance during the seedling stage in oilseed rape lines was determined by analysis of the changes in relative water content (RWC), biomass, chlorophyll a (chl-a), chlorophyll b (chl-b), chlorophyll t (chl-t) and total soluble sugars. The results of ANOVA indicated that drought stress significantly affected chl-a, chl-b and chl-t. A highly significant decline was revealed in chl-a, chl-b and chl-t content in leaves of oilseed rape under drought treatment. No significant difference was observed among the studied lines at drought stress and non-stress conditions in chl-a, chl-b and chl-t content except for L1, L2 and L3 Lines (Fig.1). Reduction of chl-a, chl-b and chl-t content, as a result of either slow synthesis or fast break down, has been considered as a typical symptom of oxidative stress (Smirnoff, 1993). The findings of the current study are in consistent with Ghobadi *et al.* (2013), Liu *et al.* (2011) results. In contrast, Ashraf *et al.* (2002) reported that drought stress did not affect chl-a, while significantly enhanced chl-b. Reduction in chlorophyll concentration is identified as a drought response mechanism in order to minimize the light absorption by chloroplasts (Pastenes *et al.*, 2005). Also, diminution of chlorophyll concentration in water deficit condition may be due to reduced absorption

of magnesium (Fig. 2) or sulfur (Fig. 3). Marschner (2012) expressed that when Mg is deficient, the chlorophyll content is diminished. Karmoket *et al.* (1991) pointed out sulfur deficit leads to diminish in chlorophyll content of leaves, stomatal aperture, and net photosynthesis.

Biomass and water potential have been used as indices to evaluate the relative drought tolerance of different *Brassica* species. Biomass production was found to be closely related with water content and water relations (Zhang *et al.*, 2014). The results of analysis of variance indicated the significant effect of drought stress on dry matter, as drought stress significantly decreased the amount of dry matter in all rapeseed lines.

While no significant difference was observed between studied lines, drought stress significantly decreased the leaf RWC (Fig. 1). Preservation of higher RWC under water deficit might be one basis for drought tolerance (Nautiyal *et al.*, 2002). However, RWC is not the only trait giving drought tolerance, as the maintenance of stomatal conductance and photosynthesis under stress appears.

According to ANOVA results, there was

highly significant difference among drought treatments in total soluble sugar content. There was significant difference among rapeseed lines in term of soluble sugar content in drought stress and non-stress conditions (Fig. 1), as soluble sugar content enhanced in all studied lines under drought stress condition. Several studies have revealed that decrease of Mg uptake, diminish the activity of enzymes involved in CO₂ fixation and increase carbohydrate accumulation in leaves (Hariadi and Shabala, 2004). Lowered absorption of the Mg and K nutrients can be the result of increase in accumulation of sugars in the leaves in comparison with the plants adequately supplied with nutrients (Cakmak *et al.*, 1994). Also Cakmak and Kirkby (2008) expressed that accumulation of carbohydrates in Mg-deficient leaves is caused directly by Mg deficiency stress and not as a consequence of reduced sink activity.

Nutrient concentration in rapeseed's leaves

The results shown in Table 1 reveal concentrations of fluoride (0.602 mg g⁻¹ DW), Nitrite (0.732 mg g⁻¹ DW), magnesium (0.477 mg g⁻¹ DW), followed by chloride (11.34 mg g⁻¹ DW), nitrate (8.30 mg g⁻¹ DW), phosphate (47.06 mg g⁻¹ DW),

Table 1. Concentration and percentage of decrease or increase of nutrients (anions and cations) under non-stress and drought stress conditions

	RWC (%)	Chl-a (mg g ⁻¹ FW)	Chl-b (mg g ⁻¹ FW)	Chl-t (mg g ⁻¹ FW)	sugar (mg g ⁻¹ DW)	Dry matter (mg)
Non-stress	91.07±2.38 ^a	7.42±0.92 ^a	2.60±0.51 ^a	10.03±1.42 ^a	38.90±8.2 ^b	3.37±0.58 ^a
Drought stress	75.80±5.41 ^b	5.90±0.98 ^b	2.08±0.52 ^b	7.98±1.46 ^b	70.04±17.0 ^a	1.77±0.35 ^b
Mean	83.43±8.76	6.66±1.21	2.34±0.58	9.01±1.76	54.47±20.56	2.57±0.94
Percentage of decrease	16.76	20.47	20.15	20.39	—	47.47
Percentage of increase	—	—	—	—	44.47	—
	Calcium (mg g ⁻¹ DW)	Ammonium (mg g ⁻¹ DW)	Potassium (mg g ⁻¹ DW)	Magnesium (mg g ⁻¹ DW)		
Non-stress	4.60±0.97 ^a	13.31±2.00 ^a	13.32±1.75 ^a	0.67±0.14 ^a		
Drought stress	2.99±0.63 ^b	7.90±1.34 ^b	10.45±1.99 ^b	0.29±0.08 ^b		
Mean	3.79±1.15	10.60±3.21	11.89±2.35	0.48±0.22		
Percentage of decrease	35.01	40.67	21.53	56.91		
Percentage of increase	—	—	—	—		
	Nitrite (mg g ⁻¹ DW)	Nitrate (mg g ⁻¹ DW)	Phosphate (mg g ⁻¹ DW)	Sulfate (mg g ⁻¹ DW)		
Non-stress	0.350±0.07 ^b	8.96±1.79 ^a	60.04±12.9 ^a	2.37±0.58 ^a		
Drought stress	1.114±0.25 ^a	7.64±1.53 ^b	34.08±7.2 ^b	1.58±0.34 ^b		
Mean	0.732±0.43	8.30±1.77	47.06±16.7	1.97±0.62		
Percentage of decrease	—	14.72	43.23	33.52		
Percentage of increase	68.59	—	—	—		

Each value represents the mean ± SD. The same latter within columns are not significantly different at P d" 0.05 as determined by Duncan's multiple range test.

sulfate ($1.97 \text{ mg g}^{-1} \text{ DW}$), ammonium ($10.60 \text{ mg g}^{-1} \text{ DW}$), potassium ($11.89 \text{ mg g}^{-1} \text{ DW}$) and calcium ($3.79 \text{ mg g}^{-1} \text{ DW}$) in leaf dry matter.

Phosphate ($60.35 \text{ mg g}^{-1} \text{ DW}$) was the most abundant ion in leaves under non-stress condition, followed by ammonium (13.31 mg g^{-1}

DW), potassium ($13.32 \text{ mg g}^{-1} \text{ DW}$), nitrate ($8.96 \text{ mg g}^{-1} \text{ DW}$) and calcium ($4.60 \text{ mg g}^{-1} \text{ DW}$). The lowest amount of ions at non-stress conditions belonged to magnesium ($0.667 \text{ mg g}^{-1} \text{ DW}$) (Table 1).

Phosphate also was the most abundant ion in drought stress ($13.91 \text{ mg g}^{-1} \text{ DW}$), followed

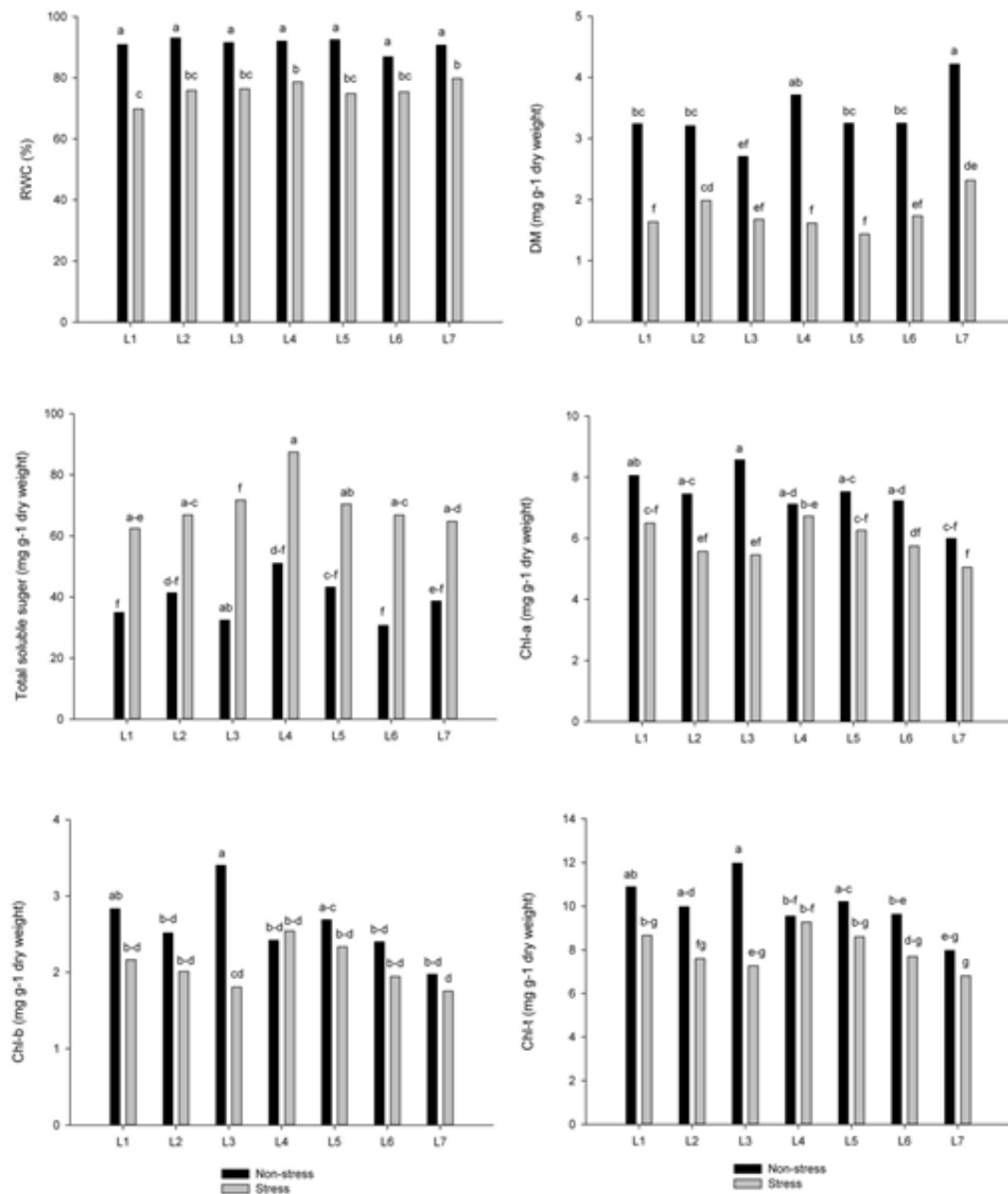


Fig. 1. Effect of the water stress on relative water content (RWC), dry matter (DM), chlorophyll a (chl-a), chlorophyll b (chl-b), chlorophyll total (chl-t) in leaf of rapeseed at non-stress and drought stress conditions. The same latter are not significantly different at $P > 0.05$ as determined by Duncan's multiple range test.

by potassium ($7.45 \text{ mg g}^{-1} \text{ DW}$), Ammonium ($7.90 \text{ mg g}^{-1} \text{ DW}$), and nitrate ($7.62 \text{ mg g}^{-1} \text{ DW}$). Also magnesium ($0.287 \text{ mg g}^{-1} \text{ DW}$) was the lowest abundant ion in drought stress condition.

Ammonium (NH_4^+), Nitrate (NO_3^-) and Nitrite (NO_2^-) uptake

N is an important source of N for plants. The results revealed a highly significant decrease in uptake of N in leaves of oilseed rape under drought treatment (Figure 3). No significant difference was observed among studied lines under drought stress and non-stress conditions in nitrate uptake except for L5 line (Fig. 2). Drought stress significantly decreased the nitrate uptake (Table 1). Under non-stress condition, higher transpiration rates results in more nutrient uptake in rapeseed plants. As shown in Table 1, Water deficit caused a 40.67% reduction in ammonium uptake compared to well-watered plants.

The results of analysis of variance indicated that stress effect were highly significant for nitrite. Highly significant difference was observed among lines under drought stress and non-stress conditions. Drought stress significantly increased the nitrite uptake (Fig. 3). The results revealed a highly significant increase in amount of N in leaves of all rapeseed lines under drought treatment. Increase the amount of nitrite in drought stress (68.59%) may be due to impaired nitrification (Table 1). During nitrification, NH_4^+ is oxidized to NO_3^- and converted to NO_2^- (Hartmann *et al.*, 2013). So under water deficit, converting nitrite to NO_3^- be greatly reduced and such reduction lead to high accumulation in soil and possibility lead to an increase in nitrite amount in rapeseed's leaves. Stark and Firestone (1995) conducted controlled experiments in which availability and soil moisture were manipulated independently and revealed lessening nitrification rates with decreasing water potential; below -0.5 MPa , nitrification was substantially inhibited.

According to ANOVA results, there was a highly significant difference between drought treatments for N uptake. Based on results, there was a significant difference between L5 line in term of N uptake under drought stress and non-stress conditions (Fig. 3). Overall, drought stress caused a 14.72% reduction in nitrate uptake compared to non-stress condition.

Under water deficiency condition, the

available soil N (NH_4^+ and NO_3^-) and N_2 fixation is greatly decreased and such reduction leads to low N accumulation and consequently low dry matter production and low crop yield (Pimratch *et al.*, 2013). Drought condition may diminish soil-N mineralization, therefore lowering the N availability, a reduced crop N uptake may also be attributed to a decreased transpiration rate to transport N from roots to shoots (Dijkstra *et al.*, 2015). Generally between inorganic forms of nitrogen (nitrate, nitrite and ammonium), indicated more sensitivity to soil moisture than NH_4^+ and NO_3^- showed the minimum sensitivity to drought (Table 1).

Phosphate uptake

Phosphorus is required for processes including the storage and transfer of energy, photosynthesis, transport of carbohydrates and the regulation of some enzymes (Marschner, 2012). The results of analysis of variance pointed out that drought stress treatment was highly significant for phosphate. Drought stress significantly decreased the phosphate uptake in all rapeseed lines (Fig. 3). The results of phosphate uptake in response to drought stress agree with report of Grossman and Takahashi (2001). The decrease of phosphate uptake in plants under drought stress was also reported in other studies (Kirnak *et al.*, 2002; Rodriguez *et al.*, 2010). It is generally accepted that the uptake of P by crop plants is lessened in dry soil conditions, and the translocation of P to the shoots is severely confined even under relatively mild drought stress. Grossman and Takahashi (2001) indicated that influence of drought on plant nutrition may be related to limited availability of energy for assimilation of PO_4^{3-} , because it must be converted in energy-dependent processes before these ions can be used for growth and development of plants. Also, Peuke and Rennenberg (2004) showed that influence of drought on P and PO_4^{3-} uptake may be because of lowered PO_4^{3-} mobility as a result of low moisture availability.

Sulfate uptake

Most of sulfur taken up by plant is in the form of sulfate (Wilkinson and Davies, 2002). Results exhibited that drought stress significantly affected sulfate uptake (Table 1). Under drought stress condition, the uptake of sulfate anion was significantly declined in all lines (except for L1 and L4 lines) (Fig. 3).

Several studies point to a role of this anion in the plant response to drought and salinity in reaction to the phytohormone abscisic acid (ABA), a major regulator of leaf stomatal conductance (Wilkinson and Davies, 2002). Cao *et al.* (2014) provided evidence for significant co-regulation of sulfur and ABA metabolism in *Arabidopsis* that may help to combat environment stresses. Sulfate and molecules derived from this anion such as glutathione, play important roles in the intrinsic response of plants to such abiotic stresses (Chan *et al.*, 2013). Sulfur deficiency leads to a decrease in chlorophyll content of leaves, stomatal aperture, and net photosynthesis. Moreover, sulfur deficiency inhibits protein synthesis thereby leading to chlorosis. Changes in the protein composition with a decrease of sulfur-rich proteins are a typical feature of sulfur-deficient plants. The lower sulfur content of proteins considerably diminishes their nutritional quality (Omirou *et al.*, 2009).

Potassium uptake

Potassium is a limiting macronutrient for

crop yield and quality. Maintaining K⁺ homeostasis is essential for stabilization of protein synthesis, formation of membrane potentials, enzyme activation and neutralization of negatively charged proteins (Shabala and Cuin, 2008; Dreyer and Uozumi, 2011; Mak *et al.*, 2014). The results of ANOVA pointed out that stress effect were highly significant for potassium uptake. A highly significant difference was observed among all lines at drought stress and non-stress conditions, except for L1 and L4 lines (Fig. 2). Drought stress significantly diminished K⁺ uptake (Table 1). The availability of K to the plant declines with decreasing soil water content, due to the diminishing mobility of K under these conditions. Asgharzad *et al.* (2009) indicated that ratio between water and K uptake and transfer into the shoot is important for K concentration in plant tissue. If the duration and intensity of the drought is short and low, the K concentration may even rise. However, when the duration and impact of drought increase, K concentration reduces (McWilliams, 2003). Effect of K deficit on vegetable growth is

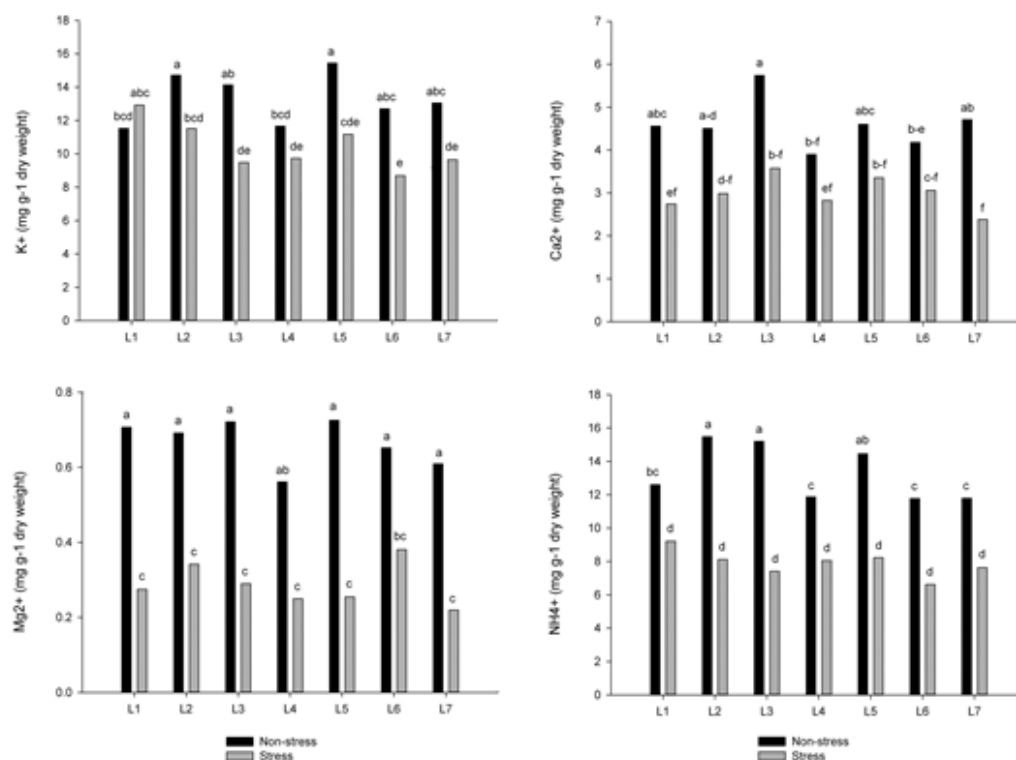


Fig. 2. Effect of the water stress on uptake of potassium (K⁺), calcium (Ca²⁺), magnesium (Mg²⁺) and ammonium (NH₄⁺) in leaf of rapeseed at non-stress and drought stress conditions. The same latter are not significantly different at P d^{0.05} as determined by Duncan's multiple range test.

described in several papers and reviews (Kanai *et al.*, 2007). Fournier *et al.* (2005) explain that K deficiency does not necessarily result in a diminished water uptake but can have also the opposite effect if the apoplastic water flow is not affected. Low K supply can interfere with and increase the uptake and mobility of Ca and Mg because of an antagonistic effect between cations as observed in many plants (Kanai *et al.*, 2007).

Calcium uptake

Calcium has been implicated in signaling the occurrence of stresses and subsequently enabling the activation of tolerance mechanisms (Choi *et al.*, 2014). Calcium is a key second messenger for drought stress responses, and drought stress-induced stomatal closure is achieved by dynamic waves of cytosolic free Ca^{2+} regulated via ABA (Dodd *et al.*, 2010). Based on ANOVA results, there was a highly significant difference between drought treatments for calcium

uptake. According to the results, calcium uptake declined in all the lines under drought stress condition (Fig. 2), although there was not a significant difference between some rapeseed lines in term of calcium uptake. The decrease of calcium uptake in plants under drought stress was also reported in other studies (Kanai *et al.*, 2007).

Although Ca uptake diminishes under drought condition (Kanai *et al.*, 2007), overall Ca accumulation is only slightly depressed in comparison with P and K. However, the low mobility of Ca renders its uptake and distribution rates limiting processes for many key plant functions. The only path for upward Ca transport is through the transpiration stream; therefore the transpiration rate is a significant determinant of Ca distribution within the plant (De Freitas *et al.*, 2011).

Any factor that inhibits root growth also diminishes Ca absorption. Calcium is responding also as an intracellular messenger on drought-

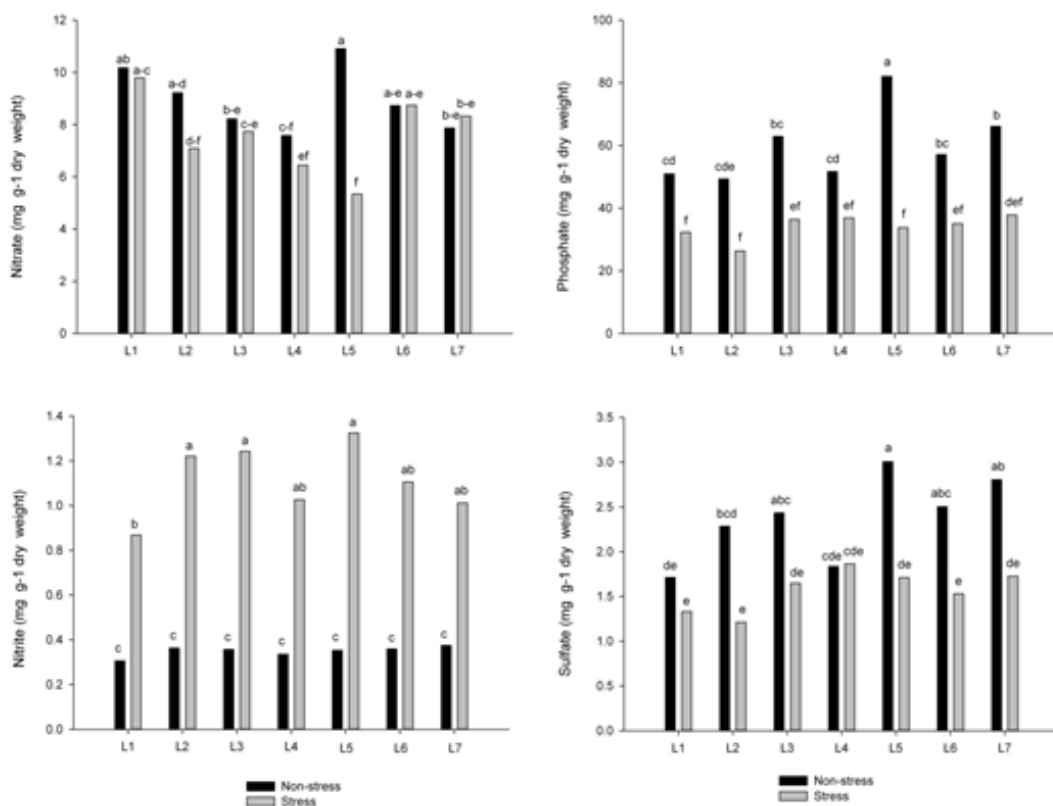


Fig. 3. Effect of water stress on uptake of nitrate, phosphate, nitrite and sulfate in leaves of rapeseed at non-stress and drought stress conditions. The same letter are not significantly different at P d'' 0.05 as determined by Duncan's multiple range test

induced proline accumulation for osmotic adjustment (Xiong *et al.*, 2006). Drought increases symptoms of Ca deficiency because water deficiency slows transpiration. A reduction in water movement within the plant reduces the amount of Ca carrying water that reaches the developing leaves and fruits (Bradfield and Guttridge, 1984). The impact of Ca deficiency increases as a consequence of drought because of several potential interactions with other factors, such as imbalances with other ions, especially with cations (Voogt, 1993), increased radiation, deficiency in micronutrients, particularly manganese, low humidity, high temperature, and CO₂-concentration (Bar Tal *et al.*, 2001), low soil pH, strong fluctuations in soil water potential (Morard *et al.*, 1996), and the available form of Ca.

Magnesium uptake

Little information is available on the effect of drought on Mg uptake in vegetable crops. Based on ANOVA, there was a highly significant difference between drought treatments for magnesium uptake. According to the results, magnesium uptake decreased in all the lines under drought stress condition (Fig. 2) When Mg is deficient, the chlorophyll content is reduced and export of carbohydrates from source to sink sites is impaired, causing a decrease of starch content (Marschner, 2012).

Inorganic nutrients such as nitrite, nitrate, ammonium (Ashraf *et al.*, 2008), phosphorus (Marschner, 2012), potassium (Fournier *et al.*, 2005; Kanai *et al.*, 2007), calcium (Marschner, 2012), sulfate (Chan *et al.*, 2013), Magnesium (Marschner, 2012) ions play multiple essential roles in plant mechanisms. This study showed that drought stress reduces nitrate, phosphate, sulfate, potassium, ammonium, calcium and magnesium uptake in rapeseed lines and concentrations of nutrients in rapeseed tissues. Also water stress increased concentration of nitrite in rapeseed leaves. Water stress affects nutrient transportation to the root and root growth. However, crop species and genotypes within a species are known to differ in their ability to take up nutrients under drought stress conditions (Garg, 2003). De Willigen and Van Noordwijk (1987) showed that, the size of the root system is the main limiting factor for P and Ca uptake and less for and K uptake. Generally,

drought reduces both nutrient uptake by the roots and transport from the roots to the shoots, because of restricted transpiration rates and impaired active transport and membrane permeability (Dijkstra *et al.*, 2015). The results of this study indicated that a reduction in soil moisture reduces the uptake of more ions, but this effect is greater for phosphate than for other ions. Lambers *et al.*, (2008) pointed out that drought can directly alter the availability of N and P by reducing their mobility in soil where the mobility of P may show greater sensitivity to soil moisture than N, particularly when N is in the nitrate form. A reduction in soil water may also diminish the dissolution and desorption of inorganic P in the soil (Belnap, 2011). Therefore, drought may reduce soil P availability more than N, at least in the short-term. Moreover, application of P fertilizer can also improve plant growth considerably under drought conditions (Garg *et al.*, 2004). In contrast, long-term effects of drought may enhance physical weathering of P and reduce biological cycling of N thereby increasing the availability of P relative to N in the soil (Delgado-Baquerizo *et al.*, 2013). Also between inorganic forms of nitrogen (nitrate, nitrite and ammonium), indicated more sensitivity to soil moisture than, and has the lowest sensitivity to drought than other anion and cations.

CONCLUSION

In conclusion, improving nutritional status of rapeseed under drought stress conditions may be essential to diminish detrimental effects of water deficit and to maintain biomass capacity of rapeseed. Also more data is needed on the combined effects of water deficit on the availability of ions and the plant uptake capacity for various nutrients. In addition, experiments are necessary to determine how changes in these elements affect the whole plant nutritional status and growth. On the other hand it is possible that drought tolerance lines will maintain high nutrient uptake under drought conditions and this ability will be a part of the reason for their drought tolerance. Therefore, more research is needed to investigate the responses of lines to the drought for nutrient uptakes at different growth stages and their contribution to drought tolerance.

REFERENCES

1. Ashraf, M., M. Arfan, M. Shahbaz, A. Ahmad and A. Jamil. Gas exchange characteristics and water relations in some elite okra cultivars under water deficit. *Photosynthetica*. 2002; **40**: 615-620.
2. Asgharzad, N. A., S. A. Bolandnazar, M. R. Neyshabouri and N. Chaparzadeh. Impact of soil sterilization and irrigation intervals on P and K acquisition by mycorrhizal onion (*Allium cepa*). *Biologia*. 2009; **64**: 512-515.
3. Bar-Tal, A., M. Keinan, Y. Oserovitz, S. Gantz, A. Avidan, I. Posalski, B. Aloni, L. Karni, A. Hazan, M. Itach, I.P. Shaham and N. Tartakovski. Relationships between blossom-end rot and water availability and Ca fertilization in bell pepper fruit production. *Acta. Hortic.* 2001; **554**: 97-105.
4. Cakmak, I., C. Hengeler and H. Marschner. Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *J. Exp. Bot.* 1994; **45**: 1245-1250.
5. Cakmak, I., and E. A. Kirkby. Role of magnesium in carbon partitioning and alleviating photo oxidative damage. *Physiologia Plantarum*. 2008; **133**: 692-704.
6. Cao, J. M., D. M. Yao, F. Lin, and M.Y. Jiang. PEG-mediated transient gene expression and silencing system in maize mesophyll protoplasts. A valuable tool for signal transduction study in maize. *Acta. Physiol. Plant.* 2014; **36**: 1271-1281.
7. Chan, K. X., M. Wirtz, S. Y. Phua, G. M. Estavillo, and B. J. Pogson. Balancing metabolites in drought: the sulfur assimilation conundrum. *Trends in plant sci.* 2013; **1**: 18-29.
8. Choi, W.G., M. Toyota, S.H. Kim, R. Hilleary, and S. Gilroy. Salt stress-induced Ca²⁺ waves are associated with rapid, longdistance root-to-shoot signaling in plants. *Proc. Natl. Acad. Sci. USA*. 2014; **111**: 6497-6502
9. Colmenero-Flores, J. M., G. Martínez, G. Gamba, N. Vázquez, D. J. Iglesias, J. Brumós, and M. Talón. Identification and functional characterization of cation/chloride cotransporters in plants. *Plant J.* 2007; **50**: 278-92.
10. De Freitas, S.T., K.A. Shackel, and E.J., Mitcham. Absciscic acid triggers whole-plant and fruitspecific mechanisms to increase fruit calcium uptake and prevent blossom end rot development in tomato fruit. *J. Exp. Bot.* 2011; **62**: 2645-2656.
11. Delgado-Baquerizo, M., F. T. Maestre, A. Gallardo, M. A. Bowker, M. D. Wallenstein, and *et al.*, Decoupling of soil nutrient cycles as a function of aridity in global drylands. *Nature*. 2013; **502**: 672-676.
12. Dijkstra, F.A., M. He, M. P. Johansen, and J. J. Harrison. Plant and microbial uptake of nitrogen and phosphorus affected by drought using ¹⁵N and ³²P tracers. *Soil Biology & Biochemistry*, 2015; 1-8.
13. Dodd, A. N., J. Kudla, and D. Sanders. The language of calcium signaling. *Annual Review of Plant Biology*. 2010; **61**: 593-620.
14. Dreyer, I., N., Uozumi. Potassium channels in plant cells. *FEBS Journal*. 2011; **278**: 4293-4303.
15. Farooq, M., A. Wahid, N. Kobayashi, D. Fujita, and S. M. A. Basra. Plant drought stress. effects, mechanisms and management. *Agron. Sustain Dev.* 2009; **29**: 185-212.
16. Fournier, J. M., A. M. Roldán, C. Sánchez, G. Alexandre, and M. Benlloch. K⁺ starvation increases water uptake in whole sunflower plants. *Plant Sci.* 2005; **168**: 823-829.
17. Garg, B. K., Nutrient uptake and management under drought: nutrient-moisture interaction. *Curr. Agric.* 2003; **27**: 1-8.
18. Garg, B. K., U. Burman, S. Kathju. The influence of phosphorus nutrition on the physiological response of moth bean genotypes to drought. *J. Plant Nutr. Soil Sci.* 2004; **167**: 503-508.
19. Ghobadi, M., Sh. Taherabadia, M. E. Ghobadi, G. R. Mohammadi, and S. Jalali-Honarmand, Antioxidant capacity, photosynthetic characteristics and water relations of sunflower (*Helianthus annuus* L.) cultivars in response to drought stress. *Industrial Crops and Products*. 2013; **50**: 29-38.
20. Ghanbari, A.A., S.H. Mousavi, A. Mousapour Gorji and I. Rao. Effect of water stress on leaves and seed of bean (*Phaseolus vulgaris* L.). *Turkish Journal of Field Crops*. 2013; **18**: 73-77
23. Grossman, A. R., and H. Takahashi. Macronutrient utilization by photosynthetic eukaryotes and the fabrics of interactions. *Annu. Rev. Plant, Physiol. Plant Mol. Biol.* 2001; **52**: 163-210.
24. Hariadi, Y., and S. Shabala. Screening broad beans (*Vicia faba*) for magnesium deficiency. II. Photosynthetic performance and leaf bioelectrical responses. *Func. Plant Biol.* 2004; **31**: 539-549.
25. Hartmann, A. A., R. L. Barnard, S. Marhan, and P. A. Niklaus. Effects of drought and N-fertilization on N cycling in two grassland soils. *Oecologia*. 2013; **171**: 705-717.

26. Jenne, E., H. Rhoades., C. Yien., O. Howe. Change in nutrient element accumulation by corn with depletion of soil moisture. *Agron. J.* 1958; **50**: 71-80.
27. Kanai, S., K. Ohkura, J. J. Adu-Gyamfi, P. K. Mohapatra, N. T. Nguyen, H. Saneoka, and K. Fujita. Depression of sink activity precedes the inhibition of biomass production in tomato plants subjected to potassium deficiency stress. *J. Exp. Bot.* 2007; **58**: 2917-2928.
28. Kirnak, H., I. Tas, C. Kaya, and D. Higgs. Effects of deficit irrigation on growth, yield and fruit quality of eggplant under semi-arid conditions. *Aust. J. Agric. Res.* 2002; **53**: 1367-1373.
29. Liu, C., Y. Liu, K. Guo, D. Fan, G. Li, Y. Zheng, L. Yu, and R. Yang. Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. *Environ. Exp. Bot.* 2011; **71**: 174-183.
30. Ma Q. F., D. W. Turner, D. Levy, and W. A. Cowling. Solute accumulation and osmotic adjustment in leaves of *Brassica* oilseeds in response to soil water deficit. *Aust. J. Agric. Res.* 2004; **55**: 939-945
31. Mak, M., M. Babla, S. C. Xu, A. O'Carrigan, X. H. Liu, Y. M. Gong, P. Holford, Z. H. Chen. Leaf mesophyll K^+ , H^+ and Ca^{2+} fluxes are involved in drought-induced decrease in photosynthesis and stomatal closure in soybean. *Environmental and Experimental Botany.*, 2014; **98**: 1-12.
32. Marschner, H. 2012. Mineral nutrition of higher plants. 3 ed. London Academic Press, pp. 651.
33. Metrohm company. 2011. Methods available from Metrohm Company for the food industry. <http://food.metrohm.com/overview/index.html>.
34. Mc Williams, D. 2003. Drought strategies for cotton, cooperative extension service circular 582, College of Agriculture and Home Economics. New Mexico State Uni. USA.
35. Morard, P., A. Pujos, A. Bernadac, and G. Bertoni. Effect of temporary calcium deficiency on tomato growth and mineral nutrition. *J. Plant Nutr.* 1996; **19**: 115-127.
36. Nautiyal, P. C., R. C. Nageswara Rao, and Y. Coshi. Moisture-deficit induced changes in leaf water content, leaf carbon exchange rate and biomass production in groundnut cultivars differing in specific leaf area. *Field Crops Res.* 2002; **74**: 67-79.
37. Omirou, M., Z. Vryzas, E. Papadopoulou-Mourkidou, and A. Economou. Dissipation rates of iprodione and thiacloprid during tomato production in greenhouse. *Food Chem.* 2009; **116**: 499-504.
38. Peuke, A.D., and H. Rennenberg. Carbon, nitrogen, phosphorus, and sulphur concentration and partitioning in beech ecotypes (*Fagus sylvatica* L.): phosphorus most affected by drought. *Trees.* 2004; **18**: 639-648.
39. Pimratch, S., N. Vorasoot, B. Toomsan, T. Kesmala, A. Patanothai, and C. C. Holbrook. Association of nitrogen fixation to water uses efficiency and yield traits of peanut. *Int. J. Plant Prod.* 2013; **7**: 225-242.
40. Rouphael, Y., M. Cardarelli, D. Schwarz, P. Franken, and G. Colla. Effects of drought on nutrient uptake and assimilation in vegetable crops. Verlag Berlin Heidelberg. 2012; 171-195.
41. Rodriguez, K. A., M. Gaczynska, and P. A. Osmulski. Molecular mechanisms of proteasome plasticity in aging. *Mech. Ageing Dev.* 2010; **131**: 144-155.
42. Smirnov, N. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol.* 1993; **125**: 27-58.
43. Stark, J. M., and M. K. Firestone. Mechanisms for soil-moisture effects on activity of nitrifying bacteria. *Appl. Environ. Microbiol.* 1995; **61**: 218-221.
44. Shabala, S., and T. A. Cuin. Potassium transport and plant salt tolerance. *Physiolgia Plantarum.* 2008; **133**: 651-669.
45. Shiranirad, A.H., Z. Bitarafan., F. Rahmani., T. Taherkhani., A. Moradi Aghdam and SH. Nasresfahani. Effect of planting date on spring rapeseed (*Brassica napus* L.) cultivars under different irrigation regimes. *Turkish Journal of Field Crops.* 2014; **19**: 153-157.
46. Tambussi, E.A., C.G., Bartoli, J., Beltrano, J.J. Guamet., J.L., Araus. Oxidative damage to thylakoid proteins in water-stressed leaves of wheat (*Triticum aestivum*). *Physiol. Plant* 2000; **108**: 398-404.
47. Vassileva, V., K. Demirevska, L. Simova-Stoilova, T. Petrova, N. Tsenov, and U. Feller. Long-term field drought affects leaf protein pattern and chloroplast ultra structure of winter wheat in a cultivar manner. *J. Agron. Crop Sci.* 2012; **198**: 104-117.
48. Voogt, W. Nutrient uptake of year round tomato crops. *Acta. Hortic.* 1993; **339**: 99-112.
49. Walker, D. J., P. Romero, A. De Hoyos, and E. Correal. Seasonal changes in cold tolerance, water relations and accumulation of cations and compatible solutes in *Atriplex halimus* L. *Environ. Exp. Bot.*, 2008; **64**: 217-224.
50. Xiong, L., R. Wang, G. Mao, and J. M. Koczan. Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant Physiol.* 2006; **142**:

- 1065-1074.
51. Zhang, X., G. Lu, W. Long, X. Zou, F. Li, T. Nishio. Recent progress in drought and salt tolerance studies in *Brassica* crops. *Breeding Sci.* 2014; **64**: 60-73.
52. Zhang, M., Z.Q. Zhu-Qun Jin, J. Zhao, G. Zhang and F. Wu, Physiological and biochemical responses to drought stress in cultivated and Tibetan wild barley. *Plant Growth Regul.* 2015; **75**: 567-574.