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ASSESSMENT OF SECONDARY METABOLISM INVOLVEMENT IN WATER STRESS TOLERANCE OF QUINOA (*CHENOPODIUM QUINOA* WILLD.) SUBJECTED TO VARYING WATER REGIMES

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Abstract

Water deficiency is a most prevalent problem which directly affects the plant growth and yield production. Keeping in view, the present study was conducted to examined the stress resistant potential of quinoa (*Chenopodium quinoa* Willd.) subjected to varying water regimes [100%, 60%, 40% and 20% field capacity (F.C.)]. Four different cultivars (V1, V2, V7 and V9) of quinoa were allowed to grow for two weeks after seed germination under normal conditions. After it, seedlings of quinoa were subjected to different levels of water stress. The required drought stress levels were maintained after 30 days of seed germination. After fifteen days of drought stress treatments, the data were collected for growth and various physio-biochemical attributes. Drought stress considerably reduced the plant growth in terms of shoot and root fresh as well as dry weights alongwith chlorophyll *a* and *b* contents and relative water contents (RWC) while a considerable increase was observed in hydrogen peroxide (H_2O_2), malondialdehyde (MDA), proline and total sugar contents in all four quinoa cultivars. Overall, it can be suggested that of all four quinoa cultivars, cv. V9 has the ability to cope with severe drought stress so it is considered as more drought tolerant and cv. V7 considered as drought sensitive particularly on the basis of plant growth.

Key words: *Chenopodium quinoa,* Water stress, Chlorophyll, Relative water contents.

Introduction

Almost all aspects of plants including physiology, anatomy, genetics and biochemistry are adversely affecting due to various environmental stresses (Ahmed *et al*., 2017). Water availability is crucial at all developmental stages of crop plants in addition to overall water applied to crop plants in obtaining maximum yield outcomes (Rollins *et al*., 2013). Less availability of water can hamper the uptake as well as utilization of nutrients to plants (He & Dijkstra, 2014). However, in some tolerant plant species, various changes (morphological and metabolic) occur in response to drought stress (Blum, 1996, Mogahdam *et al*., 2011). Under water scarce conditions, tolerance of plant is highly associates with accumulation of proline which is an amino acid (non-protein) produces in leaf tissues (Ashraf & Foolad, 2007). In addition, under stress conditions, the activity of enzymes upregulate considerably, and these enzymes are involved in various plant process such as lignifications, regulation of cell elongation, oxidative decarboxylation, glycolysis and many others (Sharma & Dubey, 2005; Almeselmani *et al*., 2006). It is well known that under water scarce conditions, activation of peroxidase enzyme may act as a regulatory mechanism by restricting cell water potential (Blokhina *et al*., 2003; Mauad *et al*., 2016). A considerable increase observed in leaf hydrogen peroxide, proline concentration and lipid peroxidation of chickpea plants under water deficiency (Gunes *et al*., 2008). It is observed that a number of plant functions either biochemical, metabolic or physiological are disturbed due to shortage of water (Levitt, 1980; Ahmed *et al*., 2017). Due to the shortage of water a remarkable reduction in growth, water contents, chlorophyll pigments and leaf fluorescence is reported (Ekmekçi *et al*., 2005; Shafiq *et al*., 2014). Most probably due to water stress conditions, absorbing capacity of roots has been loosen which leads to reduction in rate of transpiration but an increase was observed in membrane permeability (Ahmed *et al*., 2017).

Quinoa is a prehistoric grain of people belonging to the Andean region of Latin America. At present, it is cultivated in a number of countries such as Europe, Asia and America (Jacobsen, 2003; Rizzello *et al*., 2017). It is a pseudo-cereal crop and its economic and nutritive value is very high (Bhargava *et al*., 2006). Tolerating competence of quinoa plants to different environmental factors like acidity, soil salinity, flooding, drought, and frost is relatively better. In most of the regions around the world especially in China, Canada, United States, India and Europe, it has also been introducing (Bazile *et al*., 2015). Properties and qualities of its products varies due to broad range of geographical allocation with various colors such as yellow, white, black, and red etc. (Ruiz *et al*., 2014). Quinoa has practiced a revival within last two years due to its high value of nutrition (Wang & Zhu, 2015; Baldermann *et al*., 2016). Moreover, its nutritious seeds (full of starch) are used as replacement of rice and in various other products such as infant food, flakes and pancakes etc. (Li *et al*., 2016). Protein content (15%) in quinoa seeds is very high with a good sense of balance. The upper layer of quinoa seeds is rich with saponins which presents the most important anti-nutritional factor in the grain (Jurado *et al*., 2016). Keeping in view the prevailing importance of quinoa as an alternative cereal crop, the goal of the present study was to appraise the potential capacity of quinoa cultivars to varying water regimes. In addition, we explored a variety of secondary metabolites as selection criteria against water scarce conditions using quinoa plants.

Materials and Methods

To observe the tolerance capacity of quinoa (*Chenopodium quinoa* Willd.) plants, a grain food (more likely a pseudo cereal crop), various levels of water deficit conditions were applied. An experiment was arranged in the Botanical Garden situated in Government College University, Faisalabad, Pakistan. During the whole period (December, 2016 to January, 2017) of experiment, sunshine 7.05 h, RH 65.075%, and average day + night temperature 28.5°C were recorded. Seeds of four different cultivars (V9, V7, V2, and V1) were obtained from the Department of Crop Physiology, University of Agriculture Faisalabad, Pakistan. Seeds of all cultivars were dipped in water for two hours before sowing and in each pot fifteen seeds were speeded. All plastic pots were loaded with sandy-loam soil (sand 55%, clay 9.5%, silt 27.5%), pH 8.1, EC 1.89 dS m^{-1}, and saturation percentage 30% of 8 kg weight. For the maintenance of seedling numbers per treatment, thinning was done immediately after germination and six plants were remained in each pot. After three weeks of seed germination, various levels of drought stress (60%, 40% and 20% field capacity) in addition to control (100% F.C.) were applied. After fifteen days of drought stress treatments, two plants from each pot were collected and determined fresh and dry weights of shoots and roots. Remaining plants were used for the collection of data for following physico- and biochemical attributes:

Relative water contents: A 3rd leaf was removed from the apex and its fresh weight was noted. Then leaves were placed in water for three hours and their turgid weights were noted. After that, all the leaf samples were placed in an electric oven for three days and dry weights were recorded. Using the following formula, RWC were calculated.

$$\text{RWC (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Chlorophyll contents: For the determination of chlorophyll contents, a protocol proposed by Arnon (1949) was used. For this purpose, fresh leaf (0.5 g) was chopped and kept in 80% acetone for one night at -4°C. To get supernatant the extract was centrifuged at 10,000 x *g* for 30 seconds. By using a spectrophotometer, absorbance of supernatant noted at 645 and 663 nm.

Carotenoid contents: The contents of carotenoids were recorded by using the same protocol as for chlorophyll contents and OD was determined at 490 nm.

Leaf free proline: Following Bates *et al*. (1973), 3% sulfosalicylic acid solution was used to homogenized 0.5 g leaf and then filtered. Took 1 ml of the filtrate in a test tube, added 1 ml of glacial acetic acid and 1 ml acid ninhydrin solution. Then, samples were heated for 1 h at 100°C; cooled it and 4 ml toluene were added. Two separated layers were formed, the upper layer was disposed, and bottom layer was utilized for the determination of proline at 520 nm by using a spectrophotometer.

Malondialdehyde (MDA): For it, 3 ml of 1% (w/v) trichloroacetic acid (TCA) solution was used to homogenized fresh sample (0.25 g) in it and centrifuged it for 15 min at 15,000 × *g*. Took filtrate (1 ml) and added 4 ml (0.5%) TBA (prepared in 20% of TCA). The mixture of reaction was kept in a boiler at 95°C for 50 min and then cooled. Following the method of Carmak and Horst (1991), optical density was measured at 532 and 600 nm.

Hydrogen peroxide (H_2O_2): A method proposed by Velikova *et al*. (2000), 0.1% TCA solution was used as extraction medium and 0.25 g leaf tissue was homogenized in it. Then, by using centrifugation machine, all samples were centrifuged at 12,000 × *g* for 15 min. In a test tube, 1 ml of potassium iodide (KI), 0.5 ml of aliquot and 0.5 ml of phosphate buffer (potassium) were mixed. The optical density of the mixture was determined at the wavelength of 390 nm using a spectrophotometer.

Total soluble sugars: Took 0.25 g fresh leaf (0.1 g) and homogenized it in 5 ml of ethanol (80%) and shaken well for 60 min at 60°C. In a glass tube, took 100 µl of the leaf extract and anthrone reagent (3 ml) and mixed gently. Optical density was estimated at 625 nm following Yemm & Willis (1954).

Reducing sugars: For the determination of reducing sugars, a method proposed by Nelson (1944) was used. According to this method, 1 ml of leaf sample was added in 5 ml of O-toluidine (6%). Then samples were incubated for twenty minutes at 95°C and cooled the samples in an ice bath, and then OD was recorded at 630nm.

Non-reducing sugars: By applying the formula, formulated by Loomis & Shull (1937), quantity of non-reducing sugars was computed.

Non-reducing sugars = Total soluble sugars - Reducing sugars × 0.95

Statistical analysis

All the collected data was analyzed by applying two-way analysis of variance of data (ANOVA).

Results

Plant growth was highly interrupted under the deficiency of water i.e. shoot fresh and dry weights of all the selective cultivars (V1, V2, V7 and V9) were reduced considerably ($p \leq 0.001$) under varying levels of water stress (60%, 40% and 20% FC) in comparison to control (100% FC). At 100% FC, cv. V9 showed better growth as compared to the other three cultivars (V1, V2 and V7) at all levels of stress. Of all cultivars, the performance of cv. V7 was lower in terms of growth attributes and suggested as drought sensitive cultivar (Table 1; Fig. 1).

Table 1. Mean square values (ANOVA) for growth attributes, chlorophyll, carotenoids, malondialdehyde, proline, and sugars of four cultivars of quinoa (*Chenopodium quinoa* Willd.) subjected to various levels of water-deficit conditions (100%, 60%, 40% and 20% FC).

Source of variation	df	Shoot fresh wt.	Shoot dry wt.	Root fresh wt.	Root dry wt.	Shoot length	Root length
Cultivars (CV)	3	703.4***	9.332***	15.33***	0.507***	622.9***	120.7***
Drought (D)	3	482.2***	26.95***	4.937***	2.292***	1536.2***	21.66***
CV x D	9	17.9***	1.509***	0.497**	0.184***	108.3***	5.34**
Error	48	1.831	0.127	0.152	0.022	7.933	1.486
		Chl*a*	**Chl*b***	**Chl a/b**	**Total chl**	**Carotenoids**	**RWC**
Cultivars (CV)	3	0.002**	0.155***	3.156**	0.129***	0.039**	19024.3***
Drought (D)	3	0.008***	0.207***	1.89***	0.292***	0.032**	6197.1***
CVs x D	9	0.0008*	0.019ns	1.467***	0.015ns	0.019*	123.8***
Error	48	0.0003	0.013	0.287	0.0098	0.008	17.55
		Proline	**H_2O_2**	**MDA**	**Reducing sugars**	**Non-reducing sugars**	**Total sugars**
Cultivars (CV)	3	9.061***	3381361.1***	636953.1***	35656.0***	8660.1***	12623.1***
Drought (D)	3	0.008***	1244466.7***	363522.4**	1426.1*	1530.5***	504.8*
CV x D	9	0.4004*	366009.8***	95575.2ns	1737.0***	676.7**	614.9***
Error	48	0.155	19024.3	63665.7	417.5	210.4	147.8

ns = No significant; *, ** and *** = Significant at 0.05, 0.01 and 0.001 levels, respectively

Shoot and root lengths of all four cultivars were considerably declined ($p \leq 0.001$) under water stress particularly at 20% FC. Of all cultivars, shoot length of cv. V7 was lowest and cv. V9 was best at different water regimes (Table 1; Fig. 1).

Chlorophyll *a* and *b* contents were considerably reduced in all quinoa cultivars under varying water stress conditions (Table 1; Fig. 1). Of all cultivars, cultivar V1 was better and cv. V9 were lower in chlorophyll *a* contents under water-deficit conditions. However, chlorophyll *b* contents were declined significantly ($p \leq 0.001$) in cvs. V1, V2 and V9, while no change was observed in cv. V7 under water deficit conditions.

Chlorophyll *a/b* contents were affected ($p \leq 0.001$) by varying water regimes. Of all quinoa cultivars (Table 1; Fig. 1), the performance of cv. V7 was better at 20% FC as compared to all the other three cultivars (V1, V2 and V9).

Under water stress conditions, total chlorophyll contents were decreased considerably ($p \leq 0.001$) in all four cultivars (V1, V2, V7 and V9) of quinoa. It was seemed that drought stress at the rate of 20% FC showed more drastic effects on all the quinoa cultivars (Table 1; Fig. 2).

It was found that carotenoid contents were suppressed ($p \leq 0.001$) drastically at all water stress levels but the performance of cv. V2 was best at 100% but declined at 60% and 40% F.C. However, cultivar V9 was lowest at 20% F.C. (Fig. 2).

Relative water contents (RWC) were decreased ($p \leq 0.001$) in all cultivars of quinoa at all stress levels as compared to control conditions (Fig. 2). Of all the cultivars, cv. V9 showed more efficient results at all three levels of drought stress (60%, 40%, and 20% FC).

Water stress induced a significant increase ($p \leq 0.001$) in the accumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents in all quinoa cultivars. Among all quinoa cultivars, these signaling molecules were higher in cv. V1 at 40% and 20% F.C. of drought stress levels (Table 1; Fig. 2).

Leaf free proline contents were improved ($p \leq 0.001$) significantly in all quinoa cultivars on exposure to shortage of water (Table 1; Fig. 2). Of all quinoa cultivars, cv. V9 performed excellently against drought stress with respect to proline contents.

Imposition of water stress enhanced ($p \leq 0.001$) the total soluble sugars in a pattern from 100%, 60%, 40% followed by 20% F.C. It was found that at 60% FC, cv. V7 performed well as compared to the other quinoa cultivars while at 20% F.C., the response of cultivars V2 and V7 showed similar results in terms of total soluble sugars (Table 1; Fig. 2). Non-reducing and reducing sugars were increased ($p \leq 0.001$) under varying water regimes in all the quinoa cultivars. We observed more sugars (reducing and non-reducing) in cultivar V7 at all levels of drought stress.

Discussion

Shortage of water is believed as one of the most imperative growth-limiting factors which reduced the growth of plants. Plants may injure at every stage of plant growth particularly vegetative growth stage under water stress conditions (Daneshian & Zare, 2005; Tatrai *et al*., 2016). Our study revealed that a significant reduction was observed in different growth attributes as reported earlierin many plant species (Mahiwal & Sutaria, 1992; Tavakol & Pakniyat, 2007; Siddiqui *et al*., 2013; Mundim & Pringle, 2018). Well-developed system of root is considered as a strategy for desiccation avoidance in natural vegetation. In root hydraulics, morphological root constituents play a key role that may imitate various responses to water deficit conditions (Bramley *et al*., 2009). The findings of the current work were similar to Bramley *et al*., (2009). They documented that shoot used an extensive root system which may be not valuable for all the time. So, shoot growth of plants has been affected during short water supply because root system would use extra photosynthetic end products for their growth (Li *et al*., 2010).

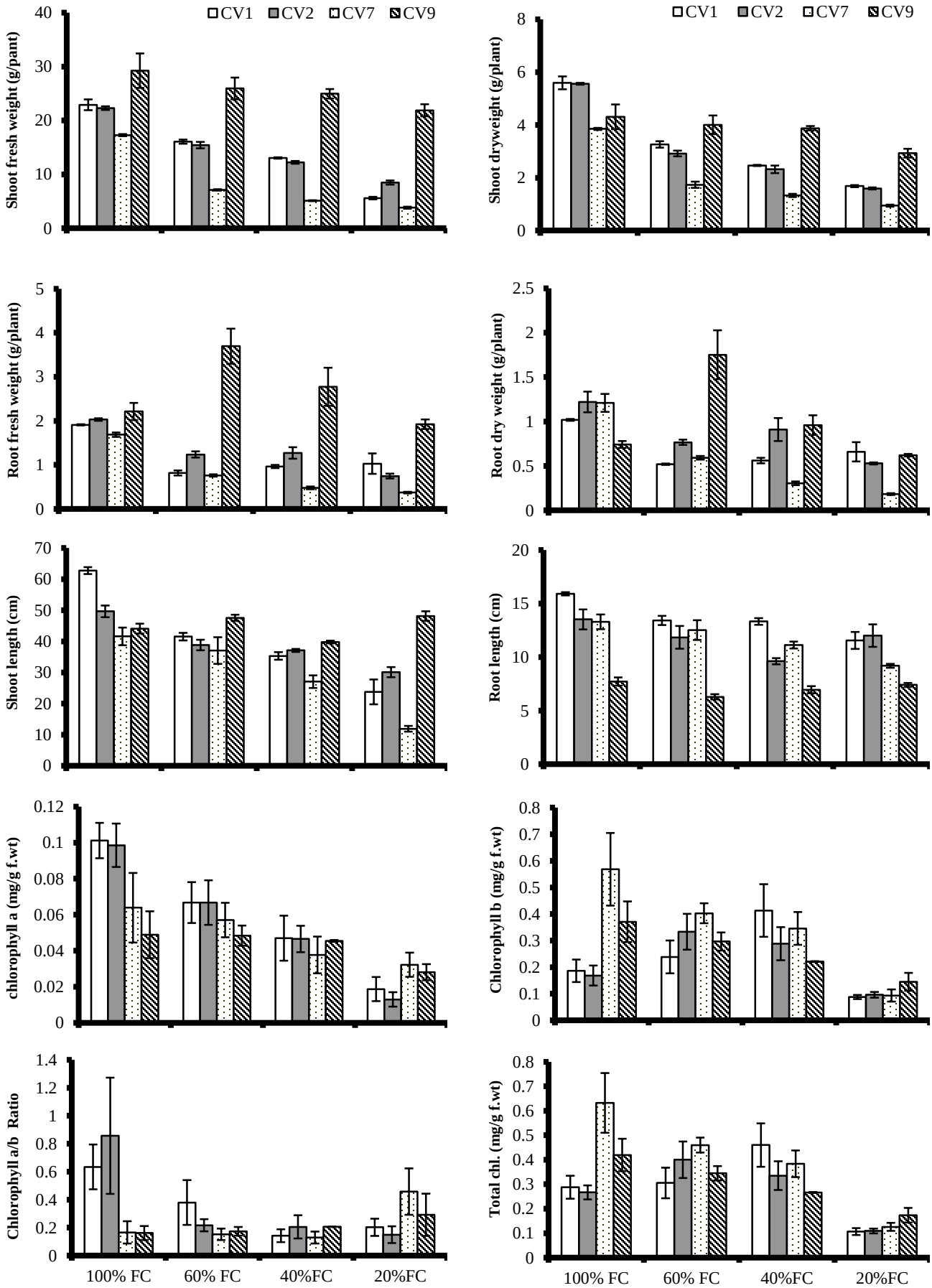

Fig. 1. Shoot fresh and dry weights, root fresh and dry weights, shoot and root lengths, chlorophyll a, b, a/b and total chlorophyll contents of four varying cultivars of quinoa (*Chenopodium quinoa* Willd.) under varying levels of drought (100, 60, 40 and 20 % FC) (Mean ± S.E).

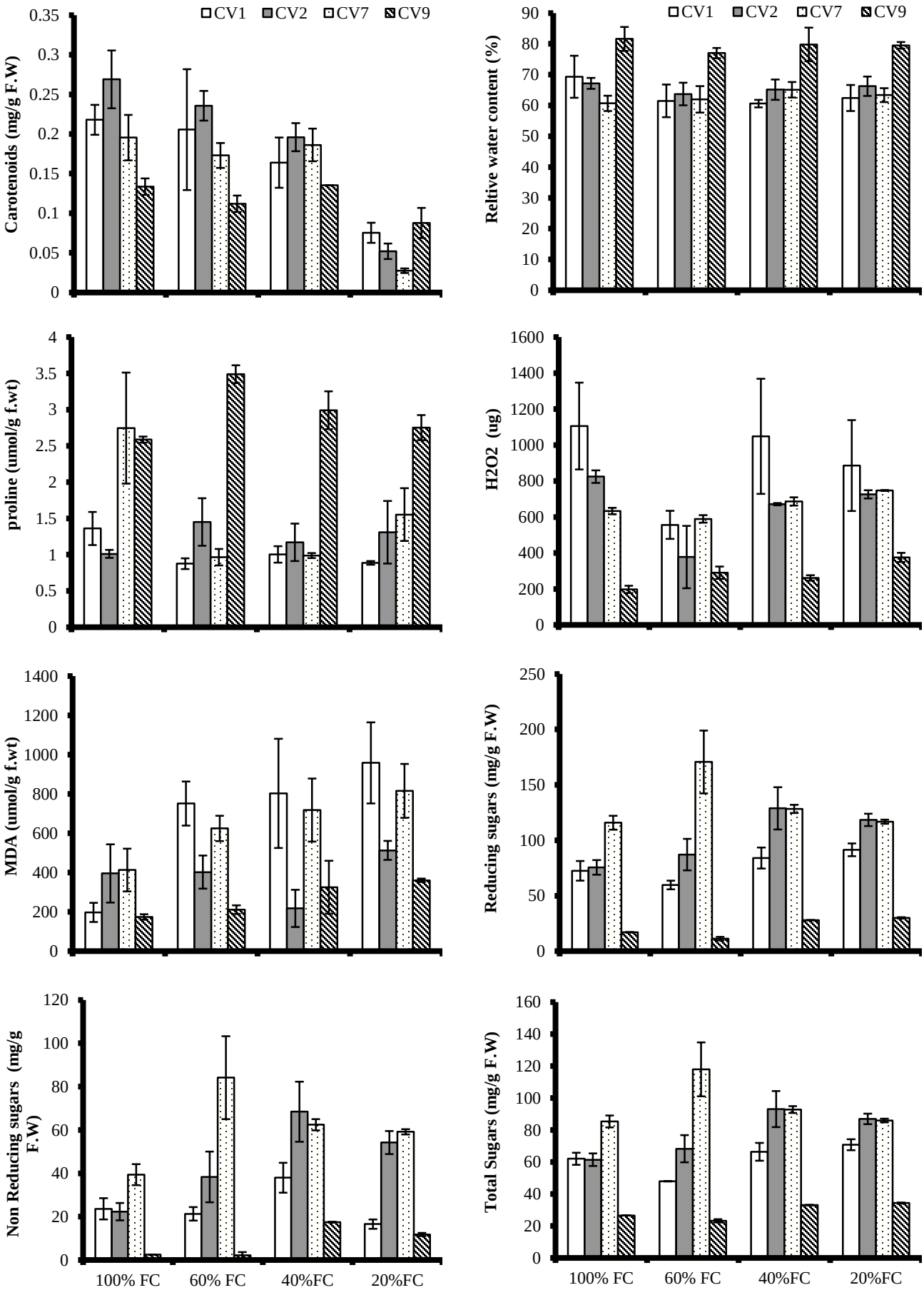

Fig. 2. Carotenoid contents, proline, H2O2, MDA, reducing sugars, non-reducing and total soluble sugars of four varying cultivars of quinoa (*Chenopodium quinoa* Willd.) under varying levels of drought (100, 60, 40 and 20 % FC) (Mean ± S.E).

Approximately in all green plants, energy is converted into chemical forms through photosynthetic pigments such as carotenoids and chlorophylls. It is well known that plants metabolism is interrupted through various abiotic stresses including drought stress because it is directly linked with photosynthetic pigments (Reza & Hassan, 2014). Under shortage of water the decline in chlorophyll contents is mainly as a result of degradation of proteins in the chloroplasts membrane which is due to the over generation of ROS (Smirnoff, 1995; Reza & Hassan, 2014; Siddiqui *et al*., 2014). It is recognized that decline in relative water content is linked with the competence of cell membrane and its ability to cope with various environmental cues including drought stress conditions. It has been examined in many plant species that reduction in RWC depends on the availability of water (Liu *et al*., 2002). Under the deficiency of water, the sustainability and penetrability of cell membrane have been decreased (Blokhina *et al*., 2003). In provisions of the physiological outcome of cellular shortage of water, RWC is possibly most suitable criterion of plant water status. The relative extent of water present in the plant tissues can be expressed through it. Similar to previous studies it has been observed in current study that under the shortage of water, RWC were decreased. The decline in RWC point out a loss of turgor due to which availability of water becomes limited for cell expansion process and subsequently plant growth and development suppressed. Relative water content is considered as a good sign of tolerance against drought stress in plants (Siddiqui *et al*., 2014; Blum, 2017).

It is well documented that an increase in contents of proline in response to water stress, in various plants helped the plants to maintain the homeostasis in leaf tissues (Slama *et al*., 2007; Mostajeran & Rahimi-Eichi, 2009; Kumar *et al.*, 2011). In the current study, it was examined that under the limited water conditions, accumulation of proline has been enhanced. In those plants which are exposed to a variety of stress conditions, proline might play an extremely valuable role in osmotic adjustment. Moreover, working as an outstanding osmolyte, proline executes three most imperative roles throughout the whole period of stress, i.e. as a signaling molecule, a metal chelator, and a protective molecule against oxidative damage. Consequently, a substantial decrease has been occurred in the activities of reactive oxygen species (Wani, 2013; Siddiqui *et al*., 2015).

Malondialdehyde (MDA) is reflected as a consistent general marker of oxidative stress that can cause membrane lipid peroxidation (Lykkesfeldt, 2007), and usually it is used in plants to review the amount of oxidative damage persuaded by varying treatments of stress. In the relative analyses, decreased level of MDA has been reported in more tolerant cultivars (Cicevan *et al*., 2016). In the current study, it has been accounted that higher rate of increase in MDA has been observed in quinoa plants at 20% F.C.

Against hazardous ecological conditions, accumulation of sugars is a self-protective strategy of plants (Munns & Tester, 2008; Ahmad & Wani, 2014). Total soluble sugars as well as non-reducing sugars were increased under shortage of water which is corresponding to the findings of Sadiq *et al*., (2017) in mungbean. Consequently, it is accomplished that sugar acts as an osmoprotectant and confers water stress tolerance in plants. In conclusion, water stress (60%, 40% and 20% F.C.) considerably diminished plant growth, chlorophyll, carotenoid and RWC, and oxidative damage of membrane by the accumulation of H_2O_2 and MDA contents. However, an increase in proline and sugar contents can be suggested as defensive system of quinoa plants under stress conditions.

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