

Research Article

Physiological Responses and Antioxidant of Wheat Cultivars in PGR -Mediated Alleviation of Drought Stress

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Abstract

Drought is one of the most important abiotic stresses and factors limiting the successful production of plant products worldwide and has adverse effects on plant growth and other metabolic processes. The role of exogenous individual or combined application of Silicon (Si) and Cycocel (CCC) (control, 3.6gL⁻¹ Si, 210mgL⁻¹ CCC, and 3.6gL⁻¹ Si + 210mgL⁻¹ CCC) on grain yield and some key physiological characteristics of wheat (*Triticum aestivum* L.) cv. Gascogen (drought-sensitive) and Aflak (drought-tolerant) was investigated under field water-stress conditions (100% and 40% field capacity). Drought stress caused a considerable reduction in biological yield, yield and yield components, relative water content and leaf water potential of both cultivars. Application of Si and CCC effectively improved these parameters in water-deficit treatments. Moreover, water-limited conditions markedly promoted the activities of key antioxidant enzymes including peroxidase, ascorbate peroxidase, catalase and superoxide dismutase as well as the levels of Malondialdehyde (MDA) and hydrogen peroxide (H₂O₂), while enhancing the accumulation of soluble sugars, potassium, magnesium and calcium in leaf tissues. Application of Si and CCC further enhanced the activities of the key antioxidant enzymes and accumulation of osmolytes, and decreased the levels of H₂O₂ and MDA in drought stressed plants; the positive effects of Si were greatest when it was applied with CCC. Synergistic effects of Si + CCC application on yield and physiological parameters were apparent compared with Si or CCC applied separately water-stress alleviation and yield improvement in the wheat cultivars by Si and CCC application was attributable to partly improved osmotic adjustment and antioxidant activity as well as to more favorable water status under stress conditions. Overall, Si and CCC application proved to have great potential in promoting grain yield of wheat in drought-prone areas.

Keywords: Drought; Membrane stability; Oxidative stress; Plant growth regulator wheat

Introduction

Wheat is a major staple food crop in the world. Increasing grain yield and improving quality are of great importance for the increasing human population [1]. Although all abiotic stresses adversely affect the wheat growth and production, water scarcity imposes the most severe effects on this crop [2]. Water scarcity adversely affects all phases of growth, most strikingly noted at the reproductive phase and grain filling, leading to fewer grains and smaller grain size in cereal crops including wheat. Impairment of assimilate partitioning and of activities of vital enzymes taking part in the synthetic processes of key carbohydrates including starch and sucrose reduces grain filling [3,4]. Drought stress is also believed to affect the uptake, transport and accumulation of key inorganic nutrients in plants [5].

According to Rao et al. [6] and Zhang et al. [7], soils are teemed with Silicon (Si). Si occurs abundantly in soils, but in the field of plant growth the other inorganic elements such as Nitrogen (N), Potassium (K), Phosphorus (P), Calcium (Ca) and Magnesium for plant growth (Mg) are more important than Si. However, Si plays an effective role in plants under stressful conditions [8-10]. E.g. Si is believed to be effective in alleviating the detrimental effects of Salinity, drought,

high temperature and heavy metals on plants [10-12]. Moreover, researches shows that Si entails useful effects in plants under water-deficit treatments, with respect to drought-induced regulation of metabolic processes and water relations [7,13,14].

However, the mechanism by which Si can effectively alleviate drought-induced harmful effects remains unknown. Various Plant Growth Regulators (PGRs) are currently used to achieve enhanced growth and production of different crops worldwide [15-17]. Of several PGRs, Cycocel (CCC) is believed to be very effective in masking the adverse effects of different abiotic and biotic stresses on crops as well as being an essential component of the signal-transduction pathways operating in plants exposed to environmental cues including drought stress [18]. Ashraf and Foolad [19] reported that CCC also has a crucial function in the mechanism of plant water stress tolerance. Exogenously applied CCC is believed to affect absorption and transport of nutrients [20], stomata regulation [21], growth and photosynthetic rate [22], chlorophyll synthesis [23] and transpiration [21].

Besides, both Si [14,24] and CCC [25,26] can promote the antioxidative defenses systems, both enzymatic and non-enzymatic,

and consequently leads to reduce damage from Reactive Oxygen Species (ROS) caused by stresses. Using Si [27] and CCC [23] also raises synthesis of osmolytes, enhancing plant tolerance versus stresses. Researchers [28,29] showed the beneficial role of osmolytes in osmoregulation. Szabados and Savoure [30] reported that the piling up of osmolytes in leaves might be effective in one or more of the above-mentioned processes and water-deficit tolerance.

Although, it has been shown that exogenous supplementation of Si or CCC can effectively promote the endurance of plants against a variety of stresses [7,18,25,31], the literature has little information on the role of Si and CCC applied in combination in alleviating drought-induced injurious effects on plants. Therefore, in the present study, we appraised the effects of exogenous Si and CCC applied individually or in combination on wheat growth and grain yield under water-deficit conditions.

Materials and Methods

Plant materials and growth conditions

Two wheat cultivars, Gascogen (drought-sensitive) and Aflak (drought-tolerant), were selected. Seeds of uniform size of both cultivars were sown in a field at the Research Farm of the College of Agriculture (altitude 1180 m a.m.s.l.), Esfahan, Iran, during the 2018-19 growing season. The crop was irrigated with good-quality irrigation water. The soil texture is loam, pH (H₂O) 7.7 and electrical conductivity (EC) 2.55dSm⁻¹.

Experimental design and treatments

The experiment was set up in a split-split-plot complete randomized block design with three replicates. Watering treatments (100% and 40% Field Capacity (FC)) were considered as main plots; foliar application of Si and CCC (control (nil), 3.6gL⁻¹ Si, 210mgL⁻¹ CCC, and 3.6gL⁻¹ Si + 210mgL⁻¹ CCC) as sub-subplots; and the two wheat cultivars as sub-subplots. The seeds were hand-sown (150kg ha⁻¹) during the first week of November in 2018. Each plot was 3m wide and 2m long. The soil was fertilized with 150kg ha⁻¹ of urea before sowing, and at mid-tillering and anthesis stages. Until the anthesis stage, all plots were irrigated to maintain 100% FC. From anthesis to ripening, water-stress treatment was initiated to maintain 40% FC, while the control plots were maintained at 100% FC. Silicon and CCC were sprayed onto the leaves of the appropriate plants at tillering and anthesis. These chemicals were sprayed for three consecutive days to ensure their uptake by the plants.

Measurements

All measurements based on fresh plant samples were done before the grain-filling stage. The fully expanded flag leaves were used for all biochemical analysis. Measurements included Relative Water Content (RWC) [32], soluble sugars [33] and soluble proteins [34]; activities of Peroxidase (POD) [35], Ascorbate Peroxidase (APX) [36], Catalase (CAT) [37] and Superoxide Dismutase (SOD) [38]; levels of hydrogen peroxide (H₂O₂) [39] and Malondialdehyde (MDA) [40]; concentrations of Ca, K and Mg by flame photometer (model 410; Corning Inc., Corning, NY, USA); and leaf water potential (Ψ_m) (PMS Instrument Company, Albany, OR, USA). At maturity, grain yield, number of grains per spike, 1000 grain weight and harvest index were measured.

Statistical analysis

Analysis of variance was performed on data for each parameter by using SAS version 9.2 software (SAS Institute, Cary, NC, USA). Significant differences among mean values were compared using Duncan's multiple range test ($P \leq 0.05$).

Results

Yield and yield components

Water stress (40% FC) significantly reduced grain number per spike by 24.65% in cv. Aflak and 38.77% in cv. Gascogen. The negative impact of water stress on number of grains per spike was alleviated by application of Si and CCC. Under water stress, foliar application of Si, CCC and Si + CCC caused an increase of 11.32%, 11.64% and 18.19%, respectively, in grain number per spike in cv. Aflak, and 8.44%, 10.91% and 13.97% in cv. Gascogen (Table 1). Furthermore, in both wheat cultivars, 1000 grain weight decreased significantly under water stress. The drought-tolerant cultivar Aflak had higher 1000-grain weight than drought-sensitive Gascogen under water stress (Table 1). The decline in 1000-grain weight was considerably less in plants supplied with Si, CCC or Si + CCC than that when these treatments were not applied. Therefore, foliar application of these treatments can significantly improve 1000-grain weight under field water-deficit conditions; maximum benefit was recorded with Si + CCC when applied under water-stress conditions to cv. Aflak, increasing 1000-grain weight by 22.90% (Table 1). Grain yield decreased significantly under water-stress conditions, by 35.55% in drought-tolerant cv. Aflak and 63.00% in drought-sensitive cv. Gascogen. However, foliar application of CCC, Si and Si + CCC caused a significant increase in grain yield under water-limited conditions. The effect of Si + CCC was greater than of Si or CCC applied separately (Table 1). With applications of Si, CCC and Si + CCC, grain yield was 18.31%, 19.71% and 31.96% higher, respectively, for cv. Aflak, and 11.03%, 18.61% and 23.36% higher for cv. Gascogen than with no foliar application under water stress (Table 1). In both cultivars, the biological yield decreased significantly under water-stress conditions; however, Si and CCC treated plants had higher biological yield than untreated plants under water stress alone. The effect of Si + CCC application on biological yield was greater than of Si or CCC applied separately (Table 1). Water stress decreased harvest index of drought-sensitive Gascogen only. Foliar application of Si + CCC significantly promoted harvest index of both wheat varieties under water-limited conditions (Table 1).

Organic substances (soluble sugars and soluble proteins) and inorganic ions

Soluble sugar concentration in the flag leaf increased significantly under water-stress conditions, by 19.09% in cv. Gascogen and 43.83% in cv. Aflak (Table 2). Plants treated with Si and CCC had significantly higher soluble sugar content than untreated plants under water stress alone. The influence of Si + CCC on soluble sugars in plants under water stress tended to be greater than of Si or CCC applied separately. The response of cultivars to Si and CCC varied significantly, with cv. Aflak more responsive; in Si, CCC and Si + CCC treatments and under water stress, soluble sugar content was 21.75%, 15.20% and 29.57% higher, respectively, in cv. Aflak, and 13.70%, 15.71% and 21.10% higher in cv. Gascogen than with no foliar application (Table 2). In both cultivars the levels of soluble proteins decreased markedly

Table 1: Influence of separate or combined application of Silicon (Si, 3.6gL⁻¹) and Cycocel (CCC, 210mgL⁻¹) on yield, yield components, biological yield and harvest index of two wheat cultivars (Gascogen and Aflak) under field water-stress and non-stress conditions.

Irrigation treatment	Chemical treatment	No. of grains per spike		1000-grain weight (g)		Grain yield (gm ⁻²)		Biological yield (gm ⁻²)		Harvest index	
		Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak
100% field capacity	0	41.01 ^b	40.56 ^b	42.00 ^a	42.00 ^a	570.00 ^a	570.87 ^a	1353.62 ^a	1300.52 ^b	42.18 ^b	43.53 ^b
	Si	41.61 ^b	43.12 ^a	42.32 ^a	42.32 ^b	570.65 ^a	570.32 ^a	1342.00 ^a	1310.23 ^{ab}	42.45 ^b	43.71 ^b
	CCC	41.54 ^b	41.32 ^b	42.81 ^a	42.81 ^a	580.23 ^a	578.32 ^a	1336.23 ^a	1315.02 ^{ab}	43.50 ^b	43.58 ^b
	Si+ CCC	43.52 ^a	43.63 ^a	43.15 ^a	43.15 ^b	584.47 ^a	582.23 ^a	1380.23 ^a	1364.02 ^a	42.38 ^b	42.30 ^b
40% field capacity	0	25.11 ^g	30.56 ^e	22.52 ^f	22.52 ^d	210.87 ^g	367.87 ^d	600.68 ^g	835.32 ^e	35.00 ^d	43.19 ^b
	Si	27.23 ^f	34.02 ^d	25.05 ^e	25.05 ^c	234.15 ^f	435.23 ^c	650.10 ^f	970.50 ^d	35.84 ^d	44.23 ^{ab}
	CCC	27.85 ^f	34.12 ^d	25.00 ^e	25.00 ^c	250.12 ^e	440.40 ^c	653.14 ^e	970.25 ^d	38.02 ^c	45.12 ^{ab}
	Si+ CCC	28.62 ^f	35.12 ^c	26.01 ^e	26.01 ^b	260.13 ^e	485.45 ^b	668.12 ^e	1000.88 ^c	38.45 ^c	48.12 ^a

For each parameter, means followed by the same letter are not significantly different at P=0.05.

Table 2: Influence of separate or combined application of Silicon (Si, 3.6gL⁻¹) and Cycocel (CCC, 210mgL⁻¹) of soluble sugars, soluble proteins and mineral nutrients in the leaves of two wheat cultivars (Gascogen and Aflak) under field water-stress and non-stress conditions Measures of sugars and minerals by dry weight, protein by fresh weight.

Irrigation treatment	Chemical treatment	Soluble sugars		Soluble proteins		Potassium		Magnesium		Calcium	
		Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak
100% FC	0	43.00 ^g	43.12 ^g	16.50 ^b	15.50 ^{bc}	48.63 ^h	47.08 ^{hi}	2.30 ^e	2.31 ^e	8.33 ^d	7.25 ^a
	Si	46.32 ^{fg}	47.32 ^f	15.85 ^{bc}	15.32 ^{bc}	48.52 ^h	48.10 ^h	2.31 ^e	2.28 ^e	8.10 ^d	7.26 ^b
	CCC	47.00 ^f	47.86 ^f	16.00 ^b	15.69 ^{bc}	52.00 ^{gh}	52.01 ^{gh}	2.30 ^e	2.29 ^e	8.21 ^d	7.15 ^a
	Si+ CCC	47.08 ^f	50.00 ^f	17.65 ^a	18.23 ^a	53.00 ^g	52.11 ^{gh}	2.29 ^e	2.31 ^e	8.15 ^d	7.28 ^b
40% FC	0	51.21 ^f	62.02 ^d	10.52 ^f	12.36 ^e	56.23 ^g	65.00 ^e	2.88 ^d	4.07 ^b	9.36 ^c	11.76 ^a
	Si	58.23 ^{de}	75.51 ^{ab}	14.02 ^d	14.36 ^d	61.85 ^f	73.10 ^c	2.85 ^d	4.10 ^b	9.45 ^c	12.95 ^a
	CCC	59.26 ^d	71.45 ^{bc}	14.00 ^d	14.32 ^d	63.15 ^d	78.00 ^a	3.02 ^c	4.65 ^a	9.27 ^c	12.64 ^a
	Si+ CCC	62.02 ^d	80.36 ^a	80.43 ^b	16.32 ^b	63.96 ^d	80.12 ^a	3.10 ^c	4.71 ^a	9.36 ^c	13.00 ^b

For each parameter, means followed by the same letter are not significantly different at P=0.05.

under water-limited conditions. Application of Si and CCC improved the soluble protein levels of water-stressed plants of both cultivars compared with plants exposed to drought stress without Si and CCC application, and the effect of Si + CCC on soluble protein content was greater than of Si or CCC applied separately. Foliar application of Si + CCC also significantly increased soluble protein content by 6.96% and 17.61%, respectively in cv. Gascogen and cv. Aflak under non-stress conditions (Table 2).

Concentrations of K, Mg and Ca increased significantly under water stress, by 38.06%, 76.19% and 62.20%, respectively, in cv. Aflak, and 15.62%, 25.21% and 12.36% in cv. Gascogen (Table 2). Drought-stressed plants fed with Si and CCC accumulated a greater concentration of K than control plants. Supplementation with CCC and Si + CCC caused a marked increase in Mg concentration in water-stressed plants compared with those receiving no foliar treatment (Table 2). Calcium concentration increased significantly in both cultivars under water stress; foliar application of Si, CCC and Si + CCC caused a further increase in this nutrient only in cv. Aflak. The concentrations of the three mineral nutrients K, Mg and Ca were greater in cv. Aflak than in cv. Gascogen under water stress conditions (Table 2).

Antioxidant enzyme activities, H₂O₂ and MDA

The activity of POD was significantly increased due to water stress, by 75.06% in cv. Aflak and 5.49% in cv. Gascogen. In both

cultivars, application of Si, CCC and Si + CCC significantly increased POD activity of water-stressed plants; the influence of Si + CCC was greater than of Si or CCC applied separately. POD was much higher in cv. Aflak than in cv. Gascogen under water-stress conditions, especially with foliar-applied Si + CCC (Table 3).

Activity of SOD rose significantly under water-stress conditions, by 34.61% in cv. Gascogen and 62.50% in cv. Aflak. Plants treated with Si or CCC had greater SOD activity than those grown solely under water limitation. The effect of Si + CCC was greater than of Si or CCC applied separately. Varietal response to Si and CCC varied significantly for SOD activity; cv. Aflak was more responsive. In addition, under normal water conditions, combined application of Si + CCC significantly promoted SOD activity relative to no foliar application in both wheat varieties (Table 3).

Activity of APX also increased in both wheat varieties under water stress, and this increase was more pronounced in cv. Aflak. Application of Si and/or CCC had no significant effect on APX activity in cv. Gascogen under either water regime, whereas in cv. Aflak, APX significantly increased with application of CCC and Si + CCC under normal water conditions and with application of Si, CCC and Si + CCC under water stress (Table 3).

In both cultivars, water stress increased the CAT activity. Application of Si or CCC supplementation had no significant effect on CAT activity in cv. Gascogen under either water-limited and

Table 3: Influence of separate or combined application of Silicon (Si, 3.6gL⁻¹) and Cycocel (CCC, 210mgL⁻¹) on activities of Peroxidase (POD), Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX) and Catalase (CAT), as well as levels of Hydrogen Peroxide (H₂O₂) and Malondialdehyde (MDA) of two wheat cultivars (Gascogen and Aflak) under field water-stress and non-stress conditions.

Irrigation treatment	Chemical treatment	POD (Um g ⁻¹ protein)		SOD (Um g ⁻¹ protein)		APX (Um g ⁻¹ protein)		CAT (Um g ⁻¹ protein)		H ₂ O ₂ (nmol g ⁻¹ FW)		MDA (mmol g ⁻¹ FW)	
		Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak
		100% FC	0	25.68 ^f	38.02 ^d	5.20 ^f	5.12 ^a	1.65 ^{ef}	1.18 ^g	3.15 ^{gh}	3.12 ^{gh}	11.71 ^g	11.23 ^g
Si	24.36 ^f		40.32 ^d	5.18 ^f	5.15 ^b	1.64 ^{ef}	1.22 ^g	3.16 ^{gh}	3.10 ^{gh}	11.02 ^g	11.00 ^g	4.00 ^h	4.14 ^{gh}
CCC	24.52 ^f		37.25 ^d	6.00 ^e	5.97 ^a	1.75 ^e	1.86 ^e	3.15 ^{gh}	3.13 ^{gh}	11.00 ^g	11.12 ^g	3.47 ^h	3.23 ^h
Si+ CCC	25.48 ^f		40.98 ^d	6.50 ^{de}	6.02 ^b	1.77 ^e	1.83 ^e	3.57 ^g	3.89 ^g	10.81 ^g	10.00 ^g	3.20 ^h	3.14 ^h
40% FC	0	27.09 ^{ef}	66.56 ^c	7.00 ^d	8.32 ^a	2.24 ^{cd}	2.87 ^c	5.11 ^{ef}	7.21 ^{cd}	56.62 ^a	42.71 ^a	17.20 ^a	12.36 ^c
	Si	30.36 ^e	76.32 ^{ab}	7.50 ^{cd}	9.12 ^a	3.85 ^c	3.62 ^{ab}	5.41 ^e	7.84 ^c	38.01 ^c	22.23 ^e	14.35 ^b	9.23 ^e
	CCC	29.39 ^e	71.65 ^b	7.50 ^{cd}	9.32 ^a	3.92 ^c	3.88 ^a	5.55 ^e	8.02 ^b	33.58 ^d	25.34 ^e	11.63 ^c	6.32 ^f
	Si + CCC	33.87 ^e	82.36 ^a	8.01 ^d	10.54 ^b	3.87 ^c	3.86 ^a	5.84 ^e	8.87 ^a	28.78 ^e	15.34 ^f	11.23 ^{cd}	6.42 ^f

For each parameter, means followed by the same letter are not significantly different at P=0.05.

Table 4: Influence of separate or combined application of Silicon (Si, 3.6gL⁻¹) and Cycocel (CCC, 210mgL⁻¹) on relative water content and leaf water potential of two wheat cultivars (Gascogen and Aflak) under field water-stress and non-stress conditions.

Irrigation treatment	Chemical treatment	Relative water content (%)		Leaf water potential (-MPa)	
		Gascogen	Aflak	Gascogen	Aflak
100% field capacity	0	82.32 ^b	83.01 ^b	1.79 ^e	32.80 ^e
	Si	82.21 ^b	84.09 ^b	1.78 ^e	29.78 ^e
	CCC	82.00 ^b	84.10 ^b	1.75 ^e	33.78 ^e
	Si+ CCC	82.00 ^b	84.00 ^b	1.73 ^e	21.68 ^{ef}
40% field capacity	0	40.13 ^f	57.02 ^c	2.99 ^a	32.43 ^{bc}
	Si	45.15 ^e	82.32 ^b	2.50 ^b	30.96 ^d
	CCC	45.20 ^e	82.23 ^b	2.51 ^b	32.97 ^d
	Si+ CCC	49.40 ^d	88.20 ^a	2.21 ^c	29.85 ^d

For each parameter, means followed by the same letter are not significantly different at P=0.05.

normal watering conditions, whereas in cv. Aflak, CAT activity increased with application of CCC and Si + CCC under drought-stress conditions (Table 3).

Levels of H₂O₂ increased markedly under water-limited conditions. Plants treated with Si and/or CCC had lower H₂O₂ levels than plants under water stress alone. Furthermore, the influence of Si + CCC application on H₂O₂ content was greater than with either Si or CCC applied separately. With application of Si, CCC and Si + CCC and under water stress, H₂O₂ content was lower than with no foliar application, in both cultivars (Table 3).

In addition, drought stress caused a significant increase in the levels of MDA in both wheat cultivars. Although the cultivars did not differ significantly from each other under normal watering, cv. Gascogen (drought-sensitive) had considerably higher levels of MDA than cv. Aflak (drought-tolerant) under water-limited conditions. Treatment with Si, CCC and Si + CCC decreased MDA levels under both non-stress and water-limited regimes in both cultivars, but the influence was more evident under water deficit (Table 3).

Relative water content and leaf water potential (Ψ_m)

Water-deficit treatments caused a marked suppression in RWC and Ψ_m in both wheat varieties. However, cv. Aflak had higher RWC and Ψ_m than cv. Gascogen under drought stress. Application of Si, CCC and Si + CCC significantly improved the RWC and Ψ_m of water-stressed plants in both cultivars (Table 4).

Discussion

Water-deficit stress (40% FC) causes reduction of duration of anthesis to grain-ripening stage, and consequently the yield of two wheat cultivars in the present study. Indeed, the importance of water presence during grain filling for yield formation of bread wheat was shown here. As a whole, Water-deficit during anthesis and grain filling reduces weight and number of grains [3-5]. Impaired grain filling was reported to be attributable to reduced partitioning of assimilates and reduced activities of key enzymes involved in sucrose and starch synthesis [5]. However, reduction of grain yield, yield components and biological yield in both wheat cultivars was lower in the presence of externally applied Si, CCC, and especially combined Si + CCC. Therefore, application of Si and CCC could improve grain yield under water-stress conditions. Similarly, Tahir et al. [41] observed that exogenous application of Si promoted grain yield in a wheat crop (by 50%) under stressful environmental conditions. Researchers (e.g. [7,10,12] have reported observation of a significant improvement in biomass under water-deficit stress, showing a promising effect of exogenous application of Si in compensating the harmful effects of water-deficit. Sonobe et al. [28]; Ahmed et al. [42] showed that these results are the same as reports for sorghum (*Sorghum bicolor*). Moreover, foliar implementation of CCC was reported to cause a considerable raise in yield of wheat under water-stress conditions [43,44].

Water-deficit stress conditions are considered to influence

physiological responses and growth of several cereal crops [45,46], and several researches state that Si could improve tolerance of plants under stressful circumstances [47-50].

Si-induced growth promotion under water-starved regimes has been reported in different crops, e.g. wheat [10,51,52], rice (*Oryza sativa*) [50,53] and soybean (*Glycine max*) [54].

Silicon is indispensable for promoting growth of several crops including cereals [55], and CCC, like several other known plant growth regulators, plays a key role in promoting plant resistance against drought stress [19]. Some reports show the vital role of exogenous supply of CCC in counteracting injurious effects of stressful environments in different plants [18]. Both CCC, as a plant growth regulator, and Si, as a mineral, are believed to regulate different physio-biochemical processes in plants including photosynthesis, stomatal regulation and ion uptake. Therefore, Si and CCC have potential functions in initiating plant growth and productivity [18,31,50,56].

Implementation of Si can considerably alleviate water-deficit damage in various plants [12,42,50,51,55-58]. Several researchers reported the advantages of soil-applied Si in counteracting both abiotic and biotic stresses [8,51,57]; while, few studies have investigated the useful effects of foliar-applied Si for counteracting these stresses [59,60].

The present investigation showed that increased activity of antioxidant enzymes such as CAT, POD, APX and SOD occurred to alleviate water-stress-induced adverse effects on wheat plants. Similar findings have been reported by Tari et al. [61] and Ashraf [14]. Molassiotis et al. [62] found that ROS-induced oxidative damage may cause oxidation of lipids and proteins. However, in the view of Moller et al. [63], a balance between ROS generation and the activities of antioxidant enzymes may ensure the extent to which oxidative damage and signaling will take place. In fact, capacity to scavenge ROS may promote drought tolerance in plants [64].

In this study, Implementation of CCC and Si raised the activity of some key enzymes taking part in the oxidative defenses system and declined the levels of MDA and H₂O₂ in drought-stressed plants. Furthermore, in cv. Aflak, the synergistic effects of Si + CCC on activity of antioxidant enzymes were greater than effects of CCC or Si applied separately, under drought-stress conditions.

Our results are similar to findings reported by other workers demonstrating that Si application to soil is very effective in mitigating the harmful effects of environmental stresses including drought [7,8,12,31]. A similar mechanism of Si and CCC in reducing drought stress is the improvement of antioxidant activity in plants under abiotic stresses [13,25]. Our findings also show that exogenously applied Si [15] and CCC [26,65] modulate the activities of vital antioxidant enzymes such as SOD and POD, and improve plant tolerance to drought stress.

A raise in mineral nutrient ions (K⁺, Mg²⁺, Ca²⁺ and Na⁺) is considered another critical mechanism for plants to endure the stress [66]. In the present study, piling up of K⁺, Ca²⁺ and Mg²⁺ occurs in the leaves of plants under water-deficit conditions, and foliar-applied Si, the same as CCC, resulted in a further increase in K⁺ levels in the

leaves of water-stressed wheat plants.

Soluble sugars also generally increase in plant tissues exposed to water-limited conditions and they are potential contributors to osmoregulation [67]. In our study, soluble sugars (as osmolytes) were considerably enhanced in the wheat leaves exposed to low water supply, more markedly in cv. Aflak (drought-tolerant), than in cv. Gascogen. Foliar application of CCC and Si further increased soluble sugar content and in cv. Aflak, and the synergistic effect of Si + CCC application was greater than of Si or CCC alone. Enhanced levels of soluble sugars are believed to have a role in stress tolerance, because soluble sugars are actively involved in protection of enzyme structure, osmoregulation, biological membrane stabilization and protection against hydroxyl radicals [67]. Nayyar and Walia [68] found that stress-resistant plants usually accumulate greater amounts of soluble sugars than stress-sensitive plants.

Leaf water potential is a potential indicator for determining plant water status, and it plays a key role in enhancing plant photosynthetic rate [69]. In the present investigation, drought-tolerant cv. Aflak maintained significantly higher Ψ_m and RWC than drought-sensitive cv. Gascogen under water-limited conditions.

Zhu et al. [70] showed that Si significantly improves the water conditions of plant leaves, which helps the plant to alleviate cellular dehydration, and therefore lower oxidative stress. Furthermore, Gong et al. [24,51] showed that application of Si to soil can improve leaf Ψ_m in plants under water-deficit conditions. Si-induced improvement in Ψ_m may be related to raise stomatal conductance and higher relative water content.

Thus, Si plays an effective role in maintaining water balance in plant tissues, most probably through higher water uptake. Isa et al. [27] reported that Si supply may improve the rigidity and strength of cell walls, thereby helping to reduce the solute leakage and stabilize the ultrastructure of biological membranes.

Silicon deposition in the cytoplasm of cells is a unique mechanism of Si in reducing abiotic stress in plants. Nonetheless, the functions of Si and CCC in water uptake and osmoregulation in plants under drought are not yet well defined. The results of this research show that osmolyte accumulation in leaves of plants treated with Si and CCC under drought stress was more prominent in drought-stressed plants receiving no Si or CCC treatment. Therefore, application of Si [7] and CCC [18] can improve the ability of plants to regulate themselves osmotically to keep high water content and leaf water potential.

Conclusion

Foliar application of Si, CCC and especially the combination Si + CCC, markedly improved grain yield and yield components of the two wheat cultivars under water-deficit. In Si, CCC and Si + CCC treatments, grain yield was 15.63%, 16.60% and 24.32% higher respectively, than with no foliar application under water stress in cv. Aflak, and 10.25%, 16.02% and 19.25% higher in cv. Gascogen. The results of the study highlight the role of Si and CCC application in regulating water-stress response of wheat, suggesting that Si and CCC are involved in physiological activities. These results showed positive effects of Si and CCC in terms of increased antioxidant activity as well as relative water content and leaf water potential. In

addition, Si and CCC stimulated the active accumulation of some osmolytes in leaves of water-stressed wheat plants, which suggests enhanced osmoregulation ability. The synergistic effects of Si + CCC application on yield and physiological parameters were greater than of Si or CCC applied separately. Therefore, proper application of Si and CCC might result in increased production of wheat, particularly in dryland areas.

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