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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, 3.6 g L-1 Si, 210 mg L-1 CCC, and 3.6 g L-1 Si + 210 mg L-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 (H_2O_2), 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 H_2O_2 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. Although all abiotic stresses adversely affect the wheat growth and production, water scarcity imposes the most severe effects on this crop. 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. Drought stress is also believed to affect the uptake, transport and accumulation of key inorganic nutrients in plants.

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. e.g. Si is believed to be effective in alleviating the detrimental effects of Salinity, drought, high temperature and heavy metals on plants [1]. 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.

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 [2]. 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. Ashraf and Foolad 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, stomata regulation, growth and photosynthetic rate, chlorophyll synthesis and transpiration.

Besides, both Si and CCC 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 and CCC also raises synthesis of osmolytes, enhancing plant tolerance versus stresses. Researchers showed the beneficial role of osmolytes in osmoregulation. Szabados and Savoure (2010) 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, the literature has little information on the role of

Si and CCC applied in combination in alleviating drought-induced injurious effects on plants [3]. 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.

METHODOLOGY

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, Esfahan, Iran, during the 2018–19 growing season. The crop was irrigated with good-quality irrigation water. The soil texture is loam, pH (H_2O) 7.7 and electrical conductivity (EC) 2.55 dS m^{-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), 3.6 g L^{-1} Si, 210 mg L^{-1} CCC, and 3.6 g L^{-1} Si+210 mg L^{-1} CCC) as sub-subplots; and the two wheat cultivars as sub-subplots. The seeds were hand-sown (150 kg ha^{-1}) during the first week of November in 2018 [4]. Each plot was 3m wide and 2m long. The soil was fertilized with 150 kg 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), soluble sugars and soluble proteins; Activities of Peroxidase (POD), Ascorbate Peroxidase (APX), Catalase (CAT) and superoxide dismutase (SOD) (Dhindsa and Matow, 1981); levels of hydrogen peroxide (H2O2) and Malondialdehyde (MDA); concentrations of Ca, K and Mg by flame photometer; and leaf water potential (Ψm). 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. Significant differences among mean values were compared using Duncan’s multiple range test (at $P \leq 0.05$).

RESULTS AND DISCUSSION

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 [5]. 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).

Table 1: Influence of separate or combined application of Silicon (Si, 3.6 g L^{-1}) and Cycocel (CCC, 210 mg L^{-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 (g m^{-2})**		**Biological yield (g m^{-2})**	
100% field capacity									
		Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak	Gascogen	Aflak
	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}

	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}
	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}
	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}
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}
	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}
	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}
	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}

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

Organic substances 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 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.

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. 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. 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).

Table 2: Influence of separate or combined application of Silicon (Si, 3.6 g L^{-1}) and Cycocel (CCC, 210 mg L^{-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	
		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}
	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}
	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}
	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}
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}
	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}
	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}
	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}

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

Antioxidant enzyme activities, H_2O_2 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.

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.

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.

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

Levels of H_2O_2 increased markedly under water-limited conditions. Plants treated with Si and/or CCC had lower H_2O_2 levels than plants under water stress alone. Furthermore, the influence of Si + CCC application on H_2O_2 content was greater than with either Si or CCC applied separately. With application of Si, CCC and Si+CCC and under water stress, H_2O_2 content was lower than with no foliar application, in both cultivars.

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).

Table 3: Influence of separate or combined application of Silicon (Si, 3.6 g L^{-1}) and Cycocel (CCC, 210 mg L^{-1}) on activities of peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT), as well as levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) of two wheat cultivars (Gascogen and Aflak) under field water-stress and non-stress conditions.

Irrigation treatment	**Chemical treatment**	**POD (U mg–1 protein)**		**SOD (U mg–1 protein)**		**APX (U mg–1 protein)**		**CAT (U mg–1 protein)**	
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}
	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}
	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}
	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}
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}
	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}
	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}
	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}
For each parameter, means followed by the same letter are not significantly different at *P=0.05*									

Relative water content and leaf water potential (Ψm)

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

Table 4: Influence of separate or combined application of Silicon (Si, 3.6 g L^{-1}) and Cycocel (CCC, 210 mg L^{-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}

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