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Full Length Article

Role of Seed Priming and Foliar Spray of Calcium in Improving Flag Leaf Growth, Grain Filling and Yield Characteristics in Wheat (*Triticum aestivum*) – A Field Appraisal

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Abstract

Flag leaf contributes greatly to grain filling in cereal crops, and its enhanced assimilate partitioning ability may be helpful in achieving greater grain yield. Calcium (Ca) is an essential element and has structural and functional roles in number of plant phenomena. Studies showed the beneficial effects of exogenously applied Ca in improving plant physiological performance but field studies on seed priming at sowing and foliar application of Ca are lacking. In this two-locational plot study, unprimed, water-primed and Ca-primed (overnight in -1.25 MPa solution of $CaCl_2.2H_2O$) wheat (*Triticum aestivum* L. cv. *Punjab-11*) seeds were sown in the field plots and foliar sprayed at grain filling (GF) stage. The flag leaf growth, gas exchange, pigment and nutrient data were obtained of the fully expanded leaf 20 days after foliar spray. Grain pigment composition was determined at 20 days after foliar spray treatments. Yield and yield components were recorded when the plants were fully matured. The results revealed that both seed priming and foliar spray of Ca exhibited superior performance for the flag leaf and grain attributes, and yielded better than untreated and water primed/sprayed plants. The foliar spray of Ca was highly effective in improving the flag leaf gas exchange properties, *chlorophyll b* and *carotenoids* as well as grain pigment contents, which contributed to grain's own growth, resulting in higher grain yield and harvest index (HI). Correlation data revealed that flag leaf and grain growth attributes were closely associated with gas exchange parameters of flag leaf and pigments contents of both the parts. There were fewer differences in the locations as priming + foliar spray interactions for transpiration rate (*E*), sub-stomatal CO_2 level (*Ci*), flag leaf and grain NO_3^--N, and number of grains per spike were present at location-I but not at location-II. On the contrary, the interactions were more strongly evident for flag leaf dry weight/area ratio, net photosynthetic rate (*Pn*), awn length and HI at location-I but weaker at location-II. In conclusion, albeit prevailing growth conditions had their own influence, foliar spray with Ca at GF stage was quite distinctly effective than seed priming in improving flag leaf growth, awn length, grain pigment and nutrient contents and economic yield by enhancing the assimilate partitioning from flag leaf and awns to developing grains.

Keywords: Foliar spray; Grain pigments; Grain filling; Awn growth; Economic yield; Wheat

Introduction

In the era of climate change, the growth and productivity of field crops are being significantly affected the world over. Field crops specialists are striving hard for getting higher yields from the existing crop varieties by mitigating the adverse effects of unpredictable climatic changes using different strategies. One of these strategies is the exogenous application of different growth promoting chemicals and modes of application may be seed pretreatment, foliar spray or via rooting media, which have proven of great significance in enhancing growth and productivity (Farooq *et al.* 2019; Batool *et al.* 2019; Rashid *et al.* 2020).

Cereal crops *e.g.*, wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.) and barley (*Hordeum vulgare* L.) are among the major sources for meeting the food demands of the world population and have therefore been extensively studied for enhancing their yields. It has been established that flag leaf in cereals makes the biggest contribution to grain development (Wahid and Rasul 2005; Farooq *et al.* 2014). It has been reported that it is the capacity rather than duration of flag leaf photosynthesis, which limits the grain filling in wheat (Tambussi *et al.* 2007; Borrill *et al.* 2015). In view of this, it is important to enhance the photosynthetic capacity of the flag leaf to accrue greater grain growth, and the exogenous application of growth enhancers may play important role in this respect, but the studies are lacking on this particular aspect.

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During spike development, the ear and grains are green and perform photosynthesis. Source-sink manipulations and carbon (^{13}C) isotope discrimination revealed a high contribution of ear (~15–29%) and awn (~4–14%) photosynthesis to grain filling (~15–20%) in durum wheat genotypes (Merah and Monneveux 2015). These authors obtained close correlation of awn (r = 0.96) and chaff (r = 0.86) ^{13}C discrimination with that of grain. Although the comparative information is limited for cereal kernels, it is reported that fruit photosynthesis in many fruit trees contributes by 20–30% to their own carbon economy at unripe stage (Wahid and Rasul 2005; Farooq *et al.* 2014). Jiang *et al.* (2017) improved the tomato fruit photosynthesis (through CO_2 assimilation and stomatal conductance) by providing light from underneath the canopy. This indicates that the photosynthetic capacity of the developing fruit can be enhanced by improving the gas exchange and photosynthetic pigment attributes. The exogenous application of growth promoting substances can be an effective approach in achieving this target.

Calcium (Ca) is an essential element for plants since it plays multiple roles in different plant phenomena. It acts as secondary messenger in signaling pathway. It also plays central role in stabilizing membrane, activate metabolic activities and may activate different enzymes (Arshi *et al*. 2006). Ca also alleviates concomitant yield reduction under stress conditions in field crops, and helps increase the ion transportation and protects the membranes (Renault 2005; Hussain *et al*. 2016). Ca priming improved germination as well as stand establishment in drought stressed and salt treated plants (Farooq *et al*. 20017; 2019). It improved photosynthesis by improving the thylakoid structural and functional properties (Hochmal *et al.* 2015). However, studies pertaining to improved flag leaf and developing grain attributes in cereal crops with the exogenous application of Ca at critically important reproductive stage are too limited and need to be established.

In view of the bulging world populace, there is a stern need to find out strategies to enhance yield of existing crop verities and understanding the mechanisms involved therein especially under ever-changing climatic conditions. Exogenous application of Ca, because of its great biological roles in plant, could be a realistic option for achieving greater grain yield under field conditions. The objective of this research was to determine the role of individual and combined application of seed priming and foliar spray on flag leaf and grain physiological attributes at the onset of grain filling (GF) stage in wheat growing at two different locations.

Materials and Methods

General experimental details

Selection of locations and meteorological conditions: The experiments were conducted at University of Agriculture, Faisalabad. Location-I was an open area in the Old Botanical Garden, with relatively more uniform soil and meteorological conditions due to higher vegetation cover but minimal trees shading. Location-II was Botany Research Area adjacent to Youngwala village, characterized by more open and relatively less uniform soil and meteorological conditions with no vegetation cover. Soil physicochemical properties from both locations are given in Table 1, while the prevailing meteorological conditions during the course of experiment in Faisalabad are given in Fig. 1. For determination of soil properties, the soil samples from both the locations were collected at 0–30 cm depth, mixed well and analyzed for physico-chemical properties (Moodie *et al.* 1959).

Source of seed, treatments application and crop management: The basic seed of wheat (*Triticum aestivum* L. cv. *Punjab-11*) was obtained from Wheat Breeder, Ayub Agricultural Research Institute, Faisalabad to perform a field plot (2 m × 3 m) study at both the locations. The aim was to investigate the effect of unpriming, and individual and combined effects of water and Ca (-1.25 MPa $CaCl_2$ solution; an optimized concentration) priming (for 24 h) and foliar spray in enhancing flag leaf and grain growth attributes. At both locations, the unprimed and primed seeds were sown in lines (22 cm row-to-row distance) at the seed rate of 125 kg/ha, on 6 November 2015 and recommended crop management practices including four irrigations, NPK @ NPK @ 120:114:60 kg/ha were applied while weeds were removed by hand. In total, there were 27 plots for nine treatments at each location. Plants were unsprayed, water sprayed and Ca sprayed at the onset of GF stage and flag leaf and grain physiological data were recorded 20 days after the foliar spray. At maturity, the plants were harvested on 25 May at location-I and on 29 May at location-II.

Crop measurements

Flag leaf and developing grain characteristics: The flag leaf characteristics were determined at 20 days after foliar spray. Area of 10 intact flag leaves per replicate from each treatment was measured as leaf length × leaf width × 0.68 (correction factor). The gas exchange attributes including net photosynthetic rate (*Pn*), transpiration rate (*E*), stomatal conductance (*gs*) and substomatal CO_2 level (*Ci*) of flag leaf and ambient CO_2 levels (*Ca*) were measured using Infra-red gas analyzer (Model LCA-4, ADC Ltd., Hoddesdon, Herts, UK). The water use efficiency was determined as *Pn*/*E* ratio. To get dry weight, the same leaves were clipped, dried in an oven at 70°C for five days and determined for dry weight. The leaf dry weight/leaf area ratio was also computed. Dried flag leaf tissue was analyzed for nutrient contents *viz.*, nitrate-N (NO_3^--N), phosphate-P (PO_4^{3-}-P), potassium (K^+), calcium (Ca^{2+}) and sulphate-S (SO_4^{2-}-S). Ten leaves were measured for the photosynthetic pigment contents.

To determine the characteristics of developing wheat grains, 20 days after foliar spray, the spikelets were separated

Table 1: Physico-chemical properties of soil collected from two locations before sowing the wheat crop

Soil characteristics	Location-I	Location-II
Color	Brown	Brown
Textural class	Loam	Clayey loam
ECe (dS/m)	2.50	2.97
SAR (mmol/L)	12.82	17.52
pH	7.10	7.53
Organic matter (%)	1.25	0.85
Sand (%)	45.56	39.21
Silt (%)	31.77	26.45
Clay (%)	22.58	34.26
Available nitrogen (mg/kg)	7.35	5.46
Available phosphorus (mg/kg)	5.33	4.96
Available potassium (mg/kg)	27.5	23.63

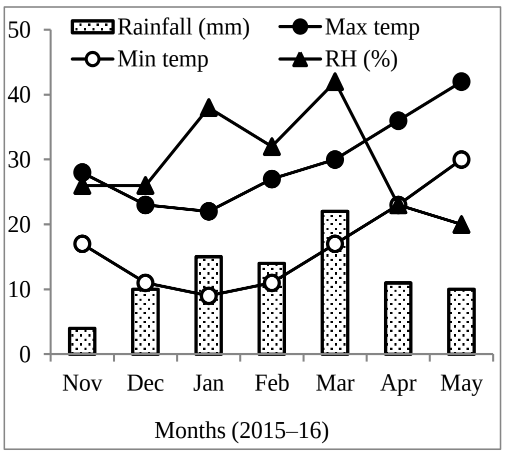

Fig. 1: Meteorological data during the period of experiment (Nov 2015 to May 2016) in Faisalabad

from the rachis, glume and lema were removed and grains were carefully removed. A 0.5 g of the grains was transferred to 80% aqueous acetone immediately after removal and measured for *chlorophyll-a* (*Chl-a*), *chlorophyll-b* (*Chl-b*) and carotenoids (*Car*). Likewise, 0.5 g of the dried grain was determined for their nutrient contents.

Spike and grain yield attributes: The spike and grain yield components were determined at maturity by counting number of spikelets per spike and number of grains per spike from five spikes per replication, while the awn length was also measured of these spikes. To determine the 1000 grain weight and grain yield data, the grains were manually extracted. The straw yield was taken as above ground dry matter including the husk weight. The harvest index (HI) was calculated as: (grain yield) ×100/straw yield.

Flag leaf and grain chemical analysis: All the analyses were performed in triplicate. The chlorophyll composition in both the plant parts was determined by the method of Arnon (1949) for chlorophylls, while for carotenoids estimation the method of Davies (1976) was followed. To accomplish this, 0.1 g of leaf sample and 0.5 g of the grain sample was extracted in 10 mL of 80% acetone, filtered, made the volume to 10 mL using 80% acetone and measured the absorbance of the extract at 663, 645 and 480 nm.

To determine the nutrient contents of the tissues (except NO_3^--N), the dried material was digested in acid mixture (HNO_3:$HClO_4$, 3:1 ratio) on a heating block by gradually increasing the temperature to 250°C until the samples became clear; filtered and made the volume up to 25 mL. K^+ and Ca^{2+} contents were measured using flame-photometer (Model 410, Sherwood Scientific Ltd., Cambridge, UK). The PO_4^{3-}-P contents were measured using spectrophotometer with molybdate-vanadate reagent (Yoshida *et al.* 1976) while SO_4^{2-}-S content was estimated with the method of Tendon *et al.* (1993). To extract NO_3^--N, the dried flag leaf and grain samples (0.1 and 0.5 g, respectively) were digested in a mixture of H_2SO_4 and H_2O_2 (1:1 ratio) using a heating block, while to measure NO_3^--N, the method of Kowalenko and Lowe (1973) was used.

Statistical analysis

The design of experiment was completely randomized factorial with three replications per treatment at both the locations. To find out the cardinal differences in the parameters investigated above, the data from each location were statistically analyzed separately for two-way variance analysis (ANOVA) to find out the presence or absence of differences and interactions in various factors using Statistics8.1 software (Table 2). In the absence of any large cardinal differences in the investigated parameters, the data from location-I were processed statistically to find significant ($P < 0.05$) differences in the treatment means by using least significant difference (LSD) test. Correlations of flag leaf growth attributes with its gas exchange, photosynthetic pigments and nutrient attributes were established using Microsoft Excel 2010 release. Similarly, correlation of pigments and nutrient contents with 1000 grain weight and grain yield per plant were also established.

Results

Variations between locations

The differences in the locations were reflected from the ANOVA carried out for data of all the parameters from both the locations. There were fewer differences in the locations as the interactions of priming and foliar spray treatment disappeared at location-II for *E*, *Ci*, flag leaf and grain NO_3^--N and number of grains per spike at location-II but were apparent at location-I. On the contrary, the interactions were strongly evident for flag leaf dry weight/area ratio, *Pn*, awn length and HI at location-I but weaker at location-II (Table 2). Such differences appeared mainly due to differences in soil properties at both locations (Table 1).

Flag leaf growth and physiological attributes

Data regarding growth parameters of wheat revealed that

Table 2: Analysis of variance (*F*-ratio) of wheat flag leaf and grain characteristics under seed priming and foliar spray treatments at two locations in Faisalabad in the year 2015–2016

Parameters	Location-I			Location-II		
	Seed priming (SP)	Foliar spray (FS)	SP × FS	Seed priming (SP)	Foliar spray (FS)	SP × FS
Flag leaf dry weight (FLDW)	22.97**	96.19**	3.30*	18.53**	65.32**	4.66*
Flag leaf area (FLA)	67.31**	41.12**	3.50*	78.65**	32.14*	4.12*
FLDW/FLA	18.21**	28.29**	5.35**	16.57**	16.24*	3.28*
Flag leaf net photosynthesis (*Pn*)	22.84**	65.02**	5.24**	17.86**	42.39**	3.27*
Flag leaf transpiration rate (*E*)	6.47**	33.36**	1.93*	3.98*	48.63**	1.18ns
Flag leaf *Pn*/*E*	2.12ns	2.55ns	0.45ns	2.25ns	3.01ns	0.56ns
Flag leaf stomatal conductance (*gs*)	3.91**	31.00**	0.18*	3.28*	24.58**	0.58**
Sub-stomatal CO_2 level (*Ci*)	2.51ns	4.03*	1.67*	1.97ns	3.22*	0.67ns
Ci/ambient CO_2 ratio	4.25ns	3.42*	0.98ns	3.36*	3.80*	0.53ns
Flag leaf *Chl-a*	2.19ns	11.76**	0.10ns	2.82ns	5.95*	0.20ns
Flag leaf *Chl-b*	12.56**	11.52**	5.23*	17.25**	8.41*	3.47*
Flag leaf *Chl-a*/*b* ratio	1.42ns	1.05ns	0.23ns	1.27ns	1.46ns	0.41ns
Flag leaf total *Chl*	4.46*	10.85**	2.17*	5.13*	12.98**	4.12**
Flag leaf *Car*	8.59**	6.06**	3.57*	6.72**	7.06**	3.40*
Flag leaf *Chl*/*Car* ratio	3.58*	3.35*	0.57ns	2.98**	4.25**	0.29ns
Flag leaf NO_3^--N	29.39**	6.06**	2.76*	18.75**	9.13**	1.06ns
Flag leaf PO_4^{3-}-P	2.93ns	5.34**	1.84ns	3.25*	6.08**	0.79ns
Flag leaf K^+	2.96*	2.78*	0.58ns	3.87*	1.68ns	0.70ns
Flag leaf Ca^{2+}	0.87ns	1.75*	0.42ns	0.50ns	2.10**	0.36ns
Flag leaf SO_4^{2-}-S	6.34**	5.10*	2.11*	9.83**	3.24*	1.87*
Grain *Chl-a*	10.13**	36.14**	3.06*	6.12*	42.56**	2.56*
Grain *Chl-b*	2.17ns	21.22**	3.06*	2.92*	15.14**	2.21*
Grain *Car*	0.58ns	29.03**	3.45*	1.32ns	8.56*	2.53*
Grain NO_3^--N	29.39**	6.06**	2.96*	23.87**	3.25*	2.01ns
Grain PO_4^{3-}-P	17.33**	3.57*	1.75*	9.89**	5.61**	1.52*
Grain K^+	27.15**	5.89*	3.10*	38.57**	3.45*	2.78*
Grain Ca^{2+}	2.63*	3.39*	0.08ns	3.09*	2.79ns	0.13ns
Grain SO_4^{2-}-S	1.62ns	0.64ns	0.11ns	1.45ns	0.81ns	0.24ns
No. of spikelets per spike	75.47**	2.07ns	0.87ns	55.20**	5.07ns	0.69ns
Awn length	32.90**	12.28**	2.98**	13.41*	45.21**	3.16*
Number of grains per spike	34.37**	15.06**	3.58*	43.33**	5.13*	1.58ns
100 grains weight	0.25ns	7.31**	0.90ns	0.71ns	5.43**	0.56ns
Grain yield per plant	31.86**	33.23**	5.92*	23.47**	42.36**	3.62*
Straw yield per plant	4.10*	3.56*	0.53ns	2.10ns	4.56**	0.29ns
Harvest index	5.25*	8.65**	2.27**	12.94**	3.65**	1.35*

Degree of freedom; SP, n = 2; FS, n = 2 and SP × FS, n = 4; Error, 18 and Total, 26
Significant at: *, $P < 0.05$; **, $P < 0.01$ and ns, $P > 0.05$

combined application of seed priming and foliar spray with Ca most significantly ($P < 0.01$) improved flag leaf dry weight, while dry weight/area ratio of flag leaf indicated a more decline at location-I indicating that the exogenous applications provided more photosynthetic area than a gain in leaf dry weight (Fig. 2).

For flag leaf gas exchange parameters, data indicated significant ($P < 0.01$) differences between seed priming and foliar spray treatments for all parameters while significant ($P < 0.05$) interaction of these factors was noted for *Pn*, *E*, *gs* and *Ci*. Results showed that *Pn*, *E*, *Pn*/*E* and *gs* were the highest while *Ci* and *Ci*/*Ca* were the lowest with seed priming + foliar spray treatments. The Ca results were followed by seed priming + foliar spray with water with few exceptions where water treatments were at par with Ca treatments (Fig. 3).

The flag leaf pigment composition revealed significant differences in seed priming and foliar spray treatments but interaction of these factors was evident for *Chl-b*, total-*Chl*, and *Car*. Compared with unprimed + unsprayed plants, combined Ca priming + foliar spray quite significantly improved the flag leaf pigments contents especially the *Chl-b* and *Car*, thereby declining *Chl-a*/*b* ratio but no specific trend for changes in total-*Chl*/*Car* ratios. Water priming + water spray was also effective in increasing the photosynthetic pigments contents but remained inferior to Ca treatments, thus suggesting the beneficial role of Ca (Table 3).

The data regarding flag leaf nutrient content indicated significant differences ($P < 0.05$) and interactions between seed priming and foliar spray treatments for flag leaf NO_3^--N and SO_4^{2-}-S content, while interaction was noted for PO_4^{3-}-P, K^+ and Ca^{2+} contents. The plants receiving combined Ca priming + foliar spray treatment exhibited greater nutrient contents in most instances as compared to those of water primed + foliar sprayed while both these treatments were superior to untreated plants except the NO_3^--N contents of unsprayed and Ca primed plants at par with Ca primed + foliar sprayed plants (Table 4).

Correlations data revealed that flag leaf dry weight and leaf area was positively correlated with all the photosynthetic pigments contents and gas exchange

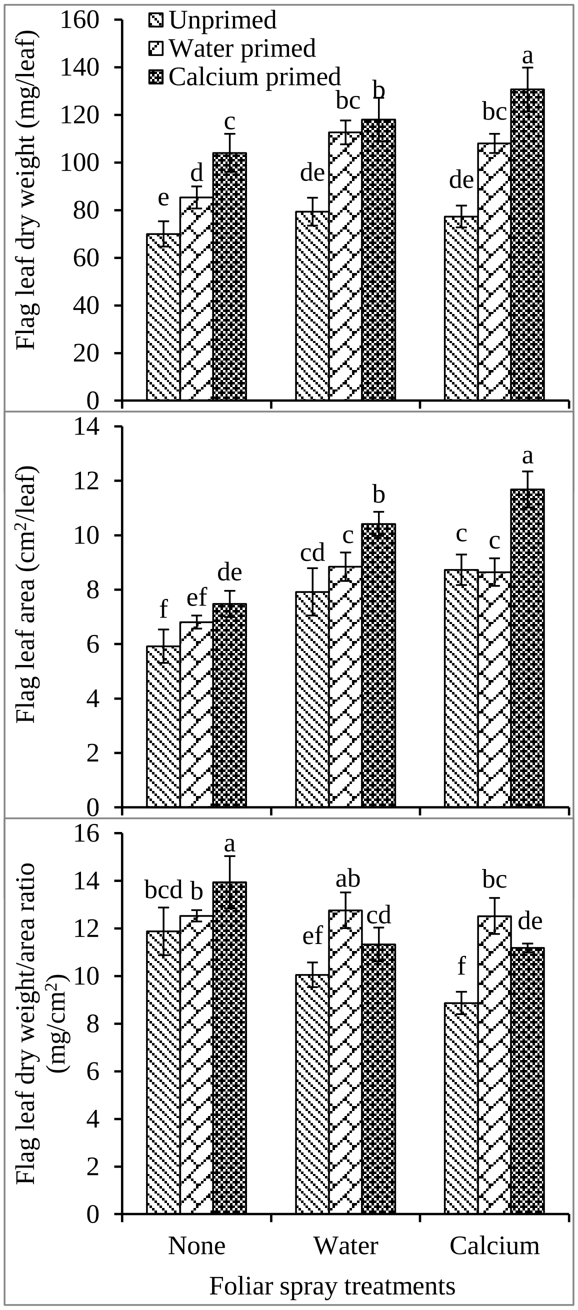

Fig. 2: Flag leaf growth characteristics from the wheat grown from unprimed, water primed and Ca primed seed, and unsprayed and foliar sprayed with water and calcium solution at grain filling stage. The columns labeled with letter show significant ($P < 0.01$) interactions of seed priming and foliar spray treatments

parameters except no correlation of flag leaf dry weight with *A/E*. On the other hand, none of the flag leaf nutrients was correlated with flag leaf dry weight while K^+, Ca^{2+} and SO_4^{2-}-S were positively correlated with flag leaf area. Moreover, flag lead dry weight/flag leaf area was correlated with none of the photosynthetic pigments, gas exchange and nutrient attributes except NO_3^--N contents (Table 5).

Grain physiological attributes

Data regarding developing grain pigment contents showed significant ($P < 0.01$) differences and interactions of seed priming and foliar spray treatments. Grain *Chl-a*, *Chl-b* and *Car* contents were noted to be the highest in the plants that received Ca priming + foliar spray and Ca priming + water spray treatments followed by the grains applied with water primed + foliar spray treatments. However, unprimed and unsprayed treatments were at the bottom (Fig. 4).

The grain nutrient data revealed significant differences in the seed priming and foliar spray treatments for all the estimated nutrients including NO_3^--N, PO_4^{3-}-P, K^+, Ca^{2+} and SO_4^{2-}-S contents while interactions of these factors was evident for NO_3^--N, PO_4^{3-}-P and K^+ only. Combined seed priming + foliar spray treatment was the most effective in enhancing the contents of all nutrients especially NO_3^--N, K^+ and Ca^{2+} in the developing grain while Ca priming + water foliar spray was the second-best treatment in improving the grain nutrient contents (Table 6). Correlations of grain *Chl-a*, *Chl-b* and *Car* data were correlated with none of the nutrient attributes except a positive correlation ($r = 0.749$; $P < 0.05$) of grain *Chl-a* with its Ca content (data not given).

Yield contributory attributes

Data regarding different grain yield contributory attributes revealed that there were significant differences between the seed priming and foliar spray treatments (Table 7), while interactions of these factors were noted for awn length, number of grains per spike, grain yield per plant and HI. With no statistical difference, the number of spikelets per spike was the highest in unsprayed + Ca primed plants, Ca primed + sprayed treatment and Ca primed + water foliar sprayed plants. Awn length was the highest in Ca primed + foliar sprayed plants followed by Ca primed + water sprayed plants and Ca + primed + water sprayed plants. Number of grains per spike was the highest in Ca primed + foliar spray treatment followed by Ca sprayed + Water primed and unsprayed + Ca primed plants while the lowest in unsprayed + unprimed, water sprayed + unprimed and Ca sprayed + unprimed plants. A 1000 grain weight and grain yield per plant was the greatest in Ca primed + sprayed plants followed by Ca sprayed + water primed treatment. Straw yield per plant was the highest in Ca primed + foliar spray treatment followed by water sprayed + Ca primed and Ca sprayed + water primed plants. Similarly, highest HI was noted in Ca primed + foliar sprayed plants followed by Ca foliar sprayed + water primed plants (Table 7). Correlation data showed that among the various yield components, awn length was positively correlated with 1000 grain weight ($r = 0.693$; $P < 0.05$), grain yield per plant ($r = 0.713$; $P < 0.05$) and HI ($r = 0.834$; $P < 0.01$); and also grain yield per plant was positively related to grain K^+ ($r = 0.803$; $P < 0.01$), Ca^{2+} ($r = 0.873$; $P < 0.01$) and SO_4^{2-}-S content ($r = 0.762$; $P < 0.05$).

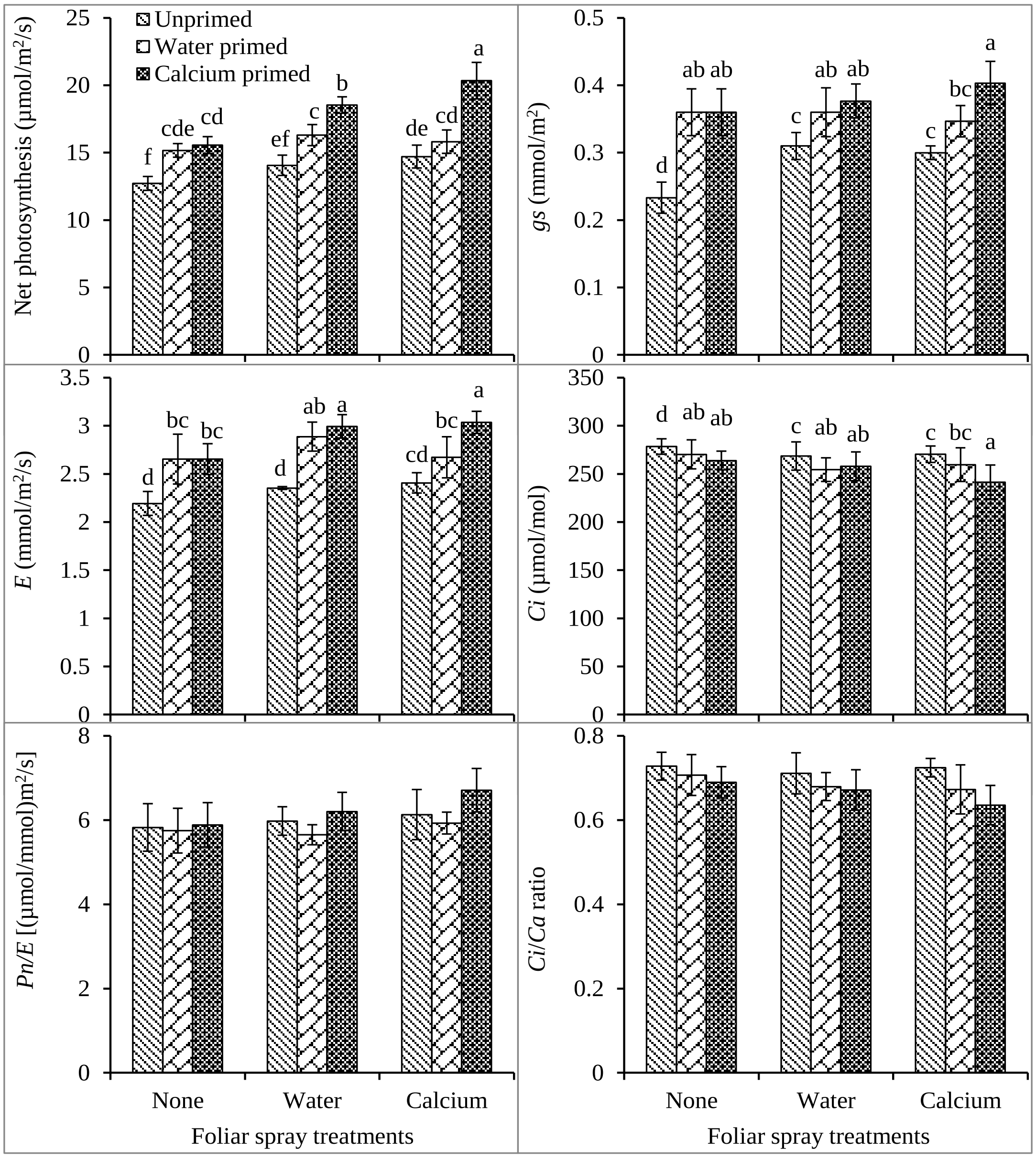

Fig. 3: Flag leaf gas exchange parameters of wheat plants grown from unprimed, water primed and calcium primed seed, and unsprayed or foliar sprayed with water and calcium solution at grain filling stage. The columns labeled with letter show significant ($P < 0.05$) interactions of seed priming and foliar spray treatments

Table 3: Flag leaf pigment composition of wheat plants grown from unprimed, water primed and calcium primed seed, and unsprayed or foliar sprayed with water and calcium solution at grain filling stage

Foliar Spray	Seed Priming	Concentration (mg/g fresh weight)					
		Chl-a	*Chl-b*	*Chl-a/b* ratio	Total *Chl*	*Car*	*Chl/Car* ratio
Unsprayed	Unprimed	2.71±0.14	1.08±0.06d	2.52±0.01	3.79±0.20d	1.21±0.09cd	3.14±0.21
	Water primed	2.89±0.15	1.18±0.05cd	2.46±0.22	4.07±0.12cd	1.19±0.06d	3.42±0.09
	Calcium primed	2.83±0.08	1.24±0.11bc	2.29±0.14	4.08±0.19cd	1.34±0.12ab	3.05±0.13
Water sprayed	Unprimed	2.94±0.14	1.14±0.04cd	2.59±0.15	4.08±0.15cd	1.31±0.06bc	3.12±0.19
	Water primed	3.10±0.30	1.24±0.04bc	2.50±0.17	4.34±0.34bc	1.30±0.04bcd	3.34±0.22
	Calcium primed	3.11±0.16	1.24±0.06bc	2.50±0.19	4.35±0.16bc	1.38±0.06ab	3.15±0.15
Calcium sprayed	Unprimed	3.14±0.28	1.22±0.05bc	2.57±0.24	4.37±0.28bc	1.28±0.05bcd	3.42±0.16
	Water primed	3.32±0.29	1.30±0.06ab	2.560.23	4.63±0.31ab	1.34±0.06ab	3.45±0.25
	Calcium primed	3.39±0.20	1.38±0.06a	2.45±0.18	4.78±0.20a	1.43±0.04a	3.33±0.22

Mean ± standard deviation: The means labeled with letter show significant ($P < 0.01$) seed priming × foliar spray interactions

Table 4: Flag leaf nutrient composition of wheat plants grown from unprimed, water primed and calcium primed seed, and unsprayed or foliar sprayed with water and calcium solution at grain filling stage

Foliar Spray	Seed Priming	Concentration (mg/g dry weight)				
		NO_3^--N	PO_4^{3-}-P	K^+	Ca^{2+}	SO_4^{2-}-S
Unsprayed	Unprimed	24.67±1.83d	9.41±0.75	31.04±1.57	11.97±1.19	7.68±0.15c
	Water primed	28.73±2.00b	9.19±1.09	33.23±2.06	12.42±0.80	7.96±0.75bc
	Calcium primed	32.21±2.15a	9.44±0.07	32.42±2.77	12.32±1.22	9.15±0.71ab
Water sprayed	Unprimed	24.67±2.26d	9.74±0.36	31.04±2.57	11.97±1.19	7.35±0.68c
	Water primed	24.53±1.46d	10.11±0.36	32.04±2.34	11.92±1.08	7.74±0.94c
	Calcium primed	28.10±1.45bc	10.04±0.33	33.07±2.81	11.91±0.94	8.36±0.87abc
Calcium sprayed	Unprimed	25.41±1.27cd	9.41±0.75	31.04±1.57	12.44±1.20	7.68±0.15c
	Water primed	25.65±1.99cd	10.18±0.53	34.27±2.28	12.74±1.06	8.42±0.64abc
	Calcium primed	32.93±1.39a	11.06±0.27	36.16±1.98	13.68±1.03	9.50±0.89a

Mean ± standard deviation: The means labeled with letter show significant ($P < 0.01$) seed priming × foliar spray interactions

Table 5: Correlation coefficient of flag leaf growth characteristics with its pigment composition, gas exchange and nutrient attributes

Variables	Dry weight	Leaf area	FLDW/FLA
Chl-a	0.972**	0.833**	0.271
Chl-b	0.855**	0.970**	-0.146
Total-*Chl*	0.965**	0.896**	0.157ns
Car	0.731*	0.769*	-0.015ns
Pn	0.924**	0.920**	0.055ns
E	0.949**	0.791*	0.296ns
Pn/E	0.500	0.799**	-0.440ns
gs	0.870**	0.712*	0.300ns
Ci	-0.949**	-0.885**	-0.137ns
NO_3^--N	0.154ns	0.591ns	-0.691*
PO_4^{3-}-P	0.264ns	0.628ns	-0.589ns
K^+	0.488ns	0.816**	-0.518ns
Ca^{2+}	0.582ns	0.836**	-0.370ns
SO_4^{2-}-S	0.416ns	0.749*	-0.510ns

*$P < 0.05$; **$P < 0.01$; ns ($P > 0.05$)

Table 6: Grain nutrient composition of wheat plants grown from unprimed, water primed and calcium primed seed, and unsprayed or foliar sprayed with water and calcium solution at grain filling stage

Foliar Spray	Seed Priming	Concentration (mg/g dry weight)				
		NO_3^--N	PO_4^{3-}-P	K^+	Ca^{2+}	SO_4^{2-}-S
Unsprayed	Unprimed	12.33±0.91d	4.14±0.16cd	5.95±0.49d	2.97±0.21	0.43±0.04
	Water primed	14.36±1.00b	4.88±0.28ab	7.70±0.57ab	3.24±0.26	0.47±0.04
	Calcium primed	16.11±1.08a	4.89±0.22ab	7.92±0.59a	3.44±0.20	0.49±0.03
Water sprayed	Unprimed	12.34±1.13d	4.00±0.20d	5.95±0.49d	2.84±0.17	0.43±0.17
	Water primed	12.27±0.73d	4.19±0.29cd	6.83±0.46c	2.94±0.26	0.42±0.11
	Calcium primed	14.05±0.73bc	4.93±0.10ab	7.71±0.34ab	3.34±0.26	0.48±0.03
Calcium sprayed	Unprimed	12.71±0.63cd	4.33±0.44cd	6.95±0.33bc	3.30±0.23	0.45±0.03
	Water primed	12.82±1.00cd	4.53±0.38bc	7.60±0.50abc	3.64±1.20	0.47±0.04
	Calcium primed	16.46±0.69a	5.02±0.30a	8.35±0.63a	3.80±0.15	0.52±0.03

Mean ± standard deviation: The means labeled with letter show significant ($P < 0.01$) seed priming × foliar spray interactions

Table 7: Yield contributory characteristics of wheat plants grown from unprimed, water primed and calcium primed seed, and unsprayed or foliar sprayed with water and calcium solution at grain filling stage

Foliar Spray	Seed Priming	No. of spikelets per spike	Awn length (cm)	No. of grains per spike	100 grains weight (g)	Grain yield per plot (kg)	Straw yield per plot (kg)	Harvest index (%)
Unsprayed	Unprimed	15.33±0.58	6.97±0.31ef	49.33±4.51e	2.75±0.14	2.43±0.19e	6.84±0.52e	35.55±1.81de
	Water primed	16.67±0.58	7.67±0.76cde	62.33±5.86bc	2.73±0.14	2.81±0.13d	7.06±0.50d	39.87±1.18cd
	Calcium primed	20.00±1.00	7.87±0.38cd	71.33±6.66ab	2.73±0.09	2.89±0.17d	7.25±0.38d	39.90±2.55cd
Water sprayed	Unprimed	15.33±0.58	6.57±0.31f	49.33±4.51e	2.84±0.20	2.47±0.23e	7.04±0.61e	35.00±1.47e
	Water primed	16.00±1.00	7.17±0.29def	59.33±5.86cd	2.80±0.08	3.00±0.13cd	7.15±0.67cd	42.10±2.93bc
	Calcium primed	19.00±1.00	8.93±0.51ab	71.33±6.66ab	2.76±0.12	3.23±0.11bc	7.79±0.26bc	41.54±2.25bc
Calcium sprayed	Unprimed	15.33±0.58	7.57±0.31cde	49.33±4.51e	2.89±0.18	2.94±0.22cd	7.25±0.37cd	40.72±4.01bc
	Water primed	17.33±0.58	8.23±0.25bc	72.67±5.51a	2.98±0.20	3.50±0.23ab	7.72±0.29ab	45.41±3.54ab
	Calcium primed	19.67±0.58	9.63±0.76a	76.00±4.36a	3.12±0.15	3.80±0.25a	7.89±0.16a	48.22±3.79a

Mean ± standard deviation.
The means labeled with letter show significant ($P < 0.01$) seed priming × foliar spray interactions

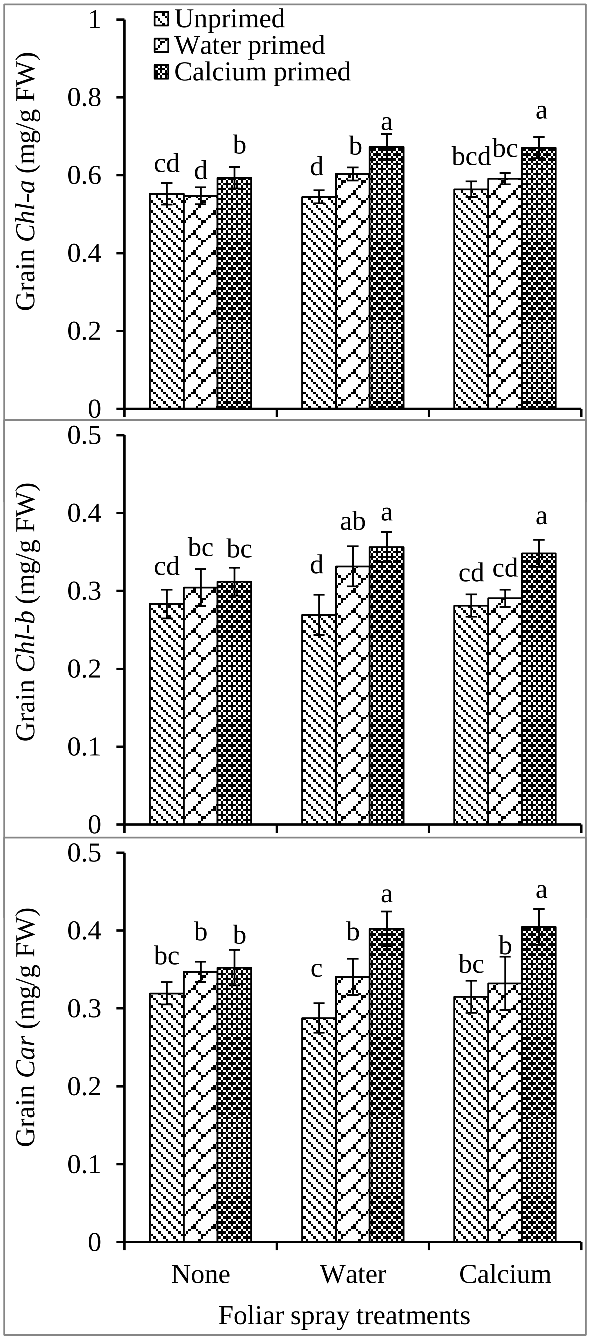

Fig. 4: Grain pigment composition of wheat plants grown from unprimed, water primed and calcium primed seed, and unsprayed or foliar sprayed with water and calcium solution at grain filling stage. The columns labeled with letter show significant ($P < 0.05$) interactions of seed priming and foliar spray treatments

Discussion

Calcium is a macronutrient and is regarded as central player in an array of plant biological phenomena (Hapler 2005; Demidchik *et al.* 2018). So, its exogenous application has been is of great interest for crop scientists in improving agronomic traits (Valadkhan *et al.* 2015). The results of this research revealed that both seed priming and foliar spray treatments improved the flag leaf dry weight and leaf area characteristics, while the ratio of both these attributes revealed that there was a greater gain in leaf area with the seed priming and foliar spray treatments of Ca followed by water (Fig. 2). This implied that exogenous Ca especially at GF stage improved the photosynthetic area more than the gain in dry weight (Hochmal *et al.* 2015). The determination of flag leaf pigment composition, especially *Chl-b* and *Car* (Table 3), gas exchange properties, especially higher *Pn* and *gs* and quite reduced *Ci* (Fig. 3), and greater nutrient content of flag leaf (Table 4) revealed that irrespective of the application mode, the Ca helped improve the wheat in the field condition. Furthermore, the presence of close correlations of flag leaf growth attributes with its pigment contents and gas exchange parameters (Table 5) further strengthened this standpoint. The exhibition of greater *Chl-b* and *Car* content is important in view of the plants growing in the field since *Chl-b* is more prone to relative adverse field conditions and Car helps tolerance against such subversive field conditions (Aderholt *et al.* 2017; Hanif and Wahid 2018).

Using ^{13}C isotope signature, it has been reported that both flag leaf and ear in cereals are major sources of assimilate partitioning to the grain growth in wheat. Inherent efficiency of plant genotype to assimilate partitioning from these parts determines the ultimate grain yield (Sanchez-Bragado *et al.* 2014; Merah and Monneveux 2015). Maintenance of greater grain pigment composition (Fig. 4) and nutrient content (Table 6) revealed the specific role of exogenous supply of Ca followed by water priming/spray in the grain filling; while there was no correlation of grain photosynthetic pigment contents with nutrient contents (data not shown). This appears to be due to the independent behavior of biosynthesis of photosynthetic pigments and nutrient partitioning from ear or flag leaf, but this aspect deserves further investigation.

The data were recorded at reproductive maturity of crop in order to quantitate the possible role of Ca and water seed priming/foliar spray treatments in spike and grain yield components (Table 7). Zoz *et al.* (2016) reported 9–32% improvement in different spike and grain yield characteristics with Ca and boron foliar spray. It was specifically seen in this study that combined application of priming + foliar spray with Ca followed by water improved awn length, number of grains per spike, grain yield per plant and HI. It is known that during grain growth, different parts of ear contribute substantially to grain filling by performing higher rate of photosynthesis in wheat cultivars (Merah and Monneveux 2015; Wang *et al.* 2016). It has been emphasized that awn has a greater contribution to grain filling in cereals due to showing critically high rate of photosynthesis and respiration (Wahid and Rasul 2005; Guo and Schnurbusch 2016; Li *et al.* 2020). Ca seed priming + foliar spray was quite effective in increasing the awn length mainly by improving awn photosynthesis (Hochmal *et al.* 2015) In our study, the awn length was appreciably higher especially with the foliar spray of Ca (Table 7), which indicated close associations with grain yield and HI as well. This revealed specific role of well-elongated awns in contributing its photoassimilates to grain filling. In addition, grain growth also appeared to be related to improved grain nutrient contents.

As evident from results, there were differences in the behavior of wheat especially in the important attributes such as *Pn*, *E*, flag leaf NO_3^--N, awn length, number of grains per spike and HI at the two locations, which are crucial players in the final grain yields (Khaliq *et al.* 2008; Hochmal *et al.* 2015). As given in Table 1, some of the interactions were strongly evident at location-I but missing at the location-II or stronger at one location than the other, although treatment applications and field operations were similar. The only responsible factor for these changes appears to be more uniform growth conditions at location-I, since strong genotype × environment interactions are considered quite crucial in the exhibition of enhanced final grain yield (George and Lundy 2019).

Conclusion

Differences in both the locations were mainly due to more uniform soil physico-chemical and meteorological properties at location-I. Flag leaf gas exchange, and pigment composition of flag leaf and grain were the major determinant of higher grain yield with Ca seed priming and foliar spray. Awn growth was one of the important spike characteristics that played a critical role in the ultimate grain yield and HI. The benefit of seed priming was carried to the reproductive growth stage since combined priming and foliar spray treatments indicated appreciably greater flag leaf and grain growth attributes in this field plot study. Further studies on the time course changes in wheat and possibly other cereals flag leaf and ear characteristics with Ca foliar spray may improve our understanding of the role of Ca in improved grain yield.

Author Contributions

NZ and AW planned the study, NZ and KS analyzed data and interpreted results; TR made illustration and interpreted results. All authors improved write up.

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