Field Efficacy of Priming Agents to Enhance Physiological Parameters of Sunflower Under Stress From Charcoal Rot (M. Phaseolina)

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Research Article

Keywords: Priming agents, resistance, environment-friendly, physiological parameters

Posted Date: May 13th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-498381/v1

Abstract

Charcoal rot (*Macrophomina phaseolina* (Tassi) Goid.) can cause significant yield losses in sunflower (*Helianthus annuus* L.) throughout the world. Fungicide treatments are often used to manage this pathogen, but due to costs and environmental impacts, alternative methods need to be explored. Priming is an adaptive strategy which enables plant defense systems to react more effectively to pathogen attack. The priming phase in plants can be achieved by stimuli from chemicals, beneficial microbes, arthropods, and abiotic stress which can induce defense systems in plants. The present study was conducted to evaluate the effect of different priming agents such as salicylic acid (SA), abscisic acid (AA), gibberellic acid (GA), and jasmonic acid (JA), as either seed or foliar treatments, on charcoal rot on the sunflower. The experiments were conducted under field conditions in two consecutive years (2017-2018). Ten seed of one susceptible (17577) and two moderately resistant (HA-259 and B-224) sunflower cultivars were treated with 2, 4 and 6 mM concentrations of the individual priming agents.. The plants were artificially inoculated with *M. phaseolina* before sowing by infesting soil, and sixty days after sowing. A number of yield parameters were quantified [head diameter (cm), 100-seed weight (g), achene per head (number), plant height (cm), stem girth (cm), shoot weight (g), root wet and dry weight (g) and chlorophyll content (mg/g)]. Among the priming agents evaluated, AA exhibited a significant impact on all yield parameters at a higher concentration (6 mM) in both susceptible and moderately resistant cultivars, followed by SA, GA, and JA. The results also revealed that seed treatment priming was more effective than foliar priming. There were statistical differences in yield parameters between both years of the study. The AA seed priming treatment showed the most promise for managing charcoal rot of sunflower. The outcome of this study will help to explore an environmentally sound and economically feasible approach for the management of charcoal rot to get sustainability in edible oil production.

Introduction

Plants under field production conditions are often exposed simultaneously to several abiotic and biotic stresses (Llorens et al., 2020). The ability to perceive the stresses and activate the proper responses is crucial for plants to grow well and be productive, and sometimes even to survive (Martínez-Medina et al., 2017). The plant immune system has been thought to be far less complex than animal immune systems (David et al., 2019). To prevent damage from pests and diseases, plants can activate their defense arsenal. Sunflower (*Helianthus annuus* L.) is a valuable, short duration (90 to 120 days), edible oilseed crop. Sunflower oil is widely used around the globe because it is rich in healthy oils, while the seed are used as animal feed (Tewari and Arora, 2018). It has great potential to meet the domestic needs of Pakistan, yet the production of sunflower has been gradually decreasing (Ahmad et al., 2017). The production of sunflower seed is affected by several abiotic and biotic stresses (Kaur et al., 2012). Diseases, caused by several plant pathogens, have resulted in a reduction in yields and economic losses, and are a threat to food security around the world. Charcoal rot of sunflower, caused by *Macrophomina phaseolina*, has been a particularly challenging disease to manage, causing 60–90% yield losses in arid areas of the world (Khan, 2007).

A wide host range of *M. phaseolina* were recorded and more than 500 wild and cultivated plant species has been reported to be the host of charcoal rot disease, these hosts including fruits, fiber crops, cereals, legumes and vegetables and also some grass species while in Pakistan 67 host species were reported (Khan, 2007).

Disease management of charcoal rot is a challenge as the disease is influenced by a wide range of variables (Katan, 2000). Furthermore, once charcoal rot is observed in the field, there are few if any management procedures that can be implemented (Gaige et al., 2010). Soil drenching with fungicides can be used for the management of charcoal rot but can have negative effects on beneficial organisms and the environment (Anis et al., 2010). Thus, seed treatments can sometimes be a better option (Kim et al., 2017).

Alternative environmentally friendly management methods would be beneficial for managing charcoal rot. One such approach would be priming the defense system of plants (Mauch-Mani et al., 2017). More recent studies suggest that systemically acquired resistance (SAR) can generate a prolonged "memory" of pathogen interaction, referred to as "priming", and that they pass the immunity to their offspring through epigenetic changes (Spoel and Dong, 2012). The "primed" state enables the plant to maintain a vigilant or alarmed status and enhances its ability to activate cellular defense responses quickly and effectively against pathogen attack (Fu and Dong, 2013). The positive effect of priming has been observed in many crops such as *Lens culinaris*, *Cicer arietinum* (Hassemi-Golezani et al., 2008), *Zea mays* (Kandil et al., 2019), *Hordeum vulgare* (Jalal et al., 2014), *Beta vulgaris* (Michalska-Klimczak et al., 2019) and *Helianthus annuus* (Asghar et al., 2019). The seed treated with priming agents can develop a better root system (Duman, 2006) in the plant that will help the plant to absorb more nutrients and improve water absorption. Seed treated with a priming agent has resulted in improved seed yield in *Phaseolus vulgaris* (Kumar et al., 2016).

The treatment of seed with priming agents has been shown to benefit the sunflower crop to mitigate stress factors such as pest damage, pathogen attack, and drought (Worrall et al., 2012). The priming agents play a vital role in protein synthesis, endosperm weakening by hydrolase activities, mobilization of storage proteins, building up of nucleic acids, cell cycle-related events, and repairing of membranes (McDonald, 2000). More production of antioxidative enzymes has been recorded in primed seed, which enables the plant to fight various biotic stresses. The positive effect of priming on seed often depends upon the duration of the priming (Hassemi-Golezani et al., 2008).

The objectives of the current study were to evaluate the efficacy of priming agents on the yield parameters of a susceptible and moderately resistant sunflower genotype under charcoal rot challenged conditions and to evaluate the effect of application methods (seed and foliar) and individual dose rates (2, 4, 6 mM) on yield parameters.

Materials And Methods

Sowing of sunflower germplasm

One susceptible (17577) and two moderately resistant (HA-259 and B-224) sunflower genotypes exhibited maximum yield (Qamar et al., 2018; Qamar et al., 2019). These germplasm were selected to determine the effect of four priming agents at three different concentrations upon charcoal rot on sunflower. Three concentrations (2, 4 and 6 mM) of SA, AA, GA, and JA were prepared for the foliar and seed applications (Table 1). For seed priming, ten seeds from each selected germplasm of sunflower were dipped for 30 minutes in each concentration, washed with sterile distilled water, air-dried on double layers of sterile filter papers, and stored at 25 ± 2 °C for 24 hours in a sterile laminar flow chamber.

Table 1
Detail of priming agents and their manufacture name.

Priming agents			**Concentrations (g/L)**			**Manufacture**	**Lot. number**
Name	**Formula**	**Molar mass (g/mol)**	**2 mM**	**4 mM**	**6 mM**		
Salicylic acid (SA)	$C_7H_6O_3$	138.121	0.28	0.56	0.84	Applichem	1R007532
Gibberellic acid (GA)	$C_{19}H_{22}O_6$	346.37	0.69	1.39	2.08	Uni-chem	GD13020469
Jasmonic acid (JA)	$C_{12}H_{18}O_3$	210.27	0.42	0.84	1.26	Sigma-aldrich	BCBE4620V
Abscisic acid (AA)	$C_{15}H_{20}O_4$	264.32	0.53	1.06	1.59	Sigma	BVC09840

Application of priming agents

The seeds were sown in the research area with 30 cm plant to plant and 60 cm row to row distance. The field experiment was arranged in a split-plot design based on a Randomized Complete Block Design (RCBD) with three replications independently to avoid pseudo-replication. The main plot consisted of four treatments with priming agents: SA, AA, GA and JA. Each treatment was divided into three subplots with 2, 4 and 6 mM concentrations of SA, AA, GA and JA, respectively.

To determine the effect of foliar application, a parallel field was prepared and seeds of selected germplasm were sown with the same plant to plant and row to row distances. Each concentration (2, 4, and 6 mM) was sprayed separately on ten days old sunflower plants and 48 hours before harvesting. A negative control plot was also prepared for comparison. The artificial inoculation of highly virulent *M. phaseolina* isolate (MIQ) (Qamar et al., 2018) with millet mass culture (4g/m) was applied in all experiments before sowing, and a spore suspension (100g mycelium/L in sterile distilled water) was applied 60 days after sowing (Keerio et al., 2014). All agronomic practices were conducted where applicable and the experiment was repeated twice.

Impact of priming agents on physiological parameters

Thirty sunflower plants from each treatment were selected randomly and harvested. Physiological parameters were recorded: 100-seed weight, achene per head, chlorophyll content, head diameter, plant height, root wet and dry weight, shoot weight, and stem girth. The effect of seed and foliar application at each concentration was recorded and compared to the control where no priming agents were applied.

Head diameter, the total number of achene per head, and 100-seed weight

The diameter (cm) of each selected sunflower plant was recorded and total numbers of seeds/achene were separated and counted (number) from individual heads (Soomro et al., 2015). 100-seeds were harvested and their weight (g) was computed (Ali et al., 2016).

Plant height, stem growth and shoot weight

The height (cm) of selected sunflower plants was recorded from the ground to the top edge of the collar disc (Soomro et al., 2015). The stem girth (cm) was measured (Soomro et al., 2015) and shoots were weighed (g) immediately after their excision (Makbul et al., 2011).

Root fresh and dry weight

The roots of the plants were separated carefully and washed with running tap water to remove soil particles and other debris. The roots were dried on a double layer of blotted paper towels and root fresh weight (g) was recorded. The roots were incubated at 80°C for 48 hours and their dry weight (g) was noted (Makbul et al., 2011).

Chlorophyll content

The fresh leaf samples (0.5 g) were plucked from the ten selected sunflower plants and ground in 10 mL of 80% acetone in sterile pestle and mortal. The mixture was centrifuged at 5000 rpm for 5 minutes and the supernatant was shifted to a new Eppendorf tube. The absorbance of the supernatant was computed at 663 and 645 nm and the chlorophyll content were measured (Farahmandfar et al., 2013).

Statistical analysis

Analysis of variance (ANOVA) was conducted to establish the differences among the independent and dependent variables. The independent variables were priming agents, concentrations, seasons, germplasm, and application methods while all physiological parameters were dependent variables. A test of homogeneity was performed on the data. The analysis was performed after having the P-value greater than 0.05, indicating the homogeneity of the data. Significant differences ($P \leq 0.05$) between the mean values were identified using Tukey's honestly significant difference (HSD) test. All statistical analyses were performed using Statistix 8.1 software (Analytical Software, 2105 Miller Landing Rd, Tallahassee, FL 32312).

Results

Head diameter (cm)

The analysis of variance revealed that all the main effects regarding head diameter were significant (Table 2). The impact of priming agents on head diameter was studied during two consecutive years in the presence of *M. phaseolina.* Priming agents were applied to three sunflower genotypes. The maximum head diameter was recorded for HA-259 in the seed (31.46 cm) and foliar application (25.28 cm) of abscisic acid at a high concentration (6mM), followed by SA, GA, and JA. A similar trend was observed with the other two genotypes (B-224 and 17577). In susceptible genotype 17577, head diameter did not exceed 11.7cm with jasmonic acid at 2mM concentration with seed application (Fig. 1).

Table 2
Analysis of variance for head diameter, seed weight, and no. of achene per head of sunflower in an experiment with two application methods, two seasons, three germplasm, four priming agents and four concentrations (total df = 575).

		***F* values**[a]		
Source of variation	df	Head diameter (cm)	Seed weight (g)	No. of achene/head
Application	1	15046.2**	9011.75**	4562.43**
Season	1	97.98**	1.01[ns]	0.00[ns]
Germplasm	2	55800.6**	5617.45**	22478.4**
Priming agent	3	4146.14**	7904.20**	679.14**
Concentration	3	4169.21**	11044.6**	5978.70**

[a]Levels of probability: ns, not significant; **, 5%.

Total number of achene per head

All the main effects regarding the number of achene per head were highly significant (Table 2). Abscisic acid significantly influenced the number of achene per head in consecutive years. When sunflower seeds were treated with AA, increasing the priming agent concentration from 2 mM to 6 mM caused the number of achene per head to increase from 951 to 1081 in the 17577 cultivar, 1313 to 1390 in the B-224 cultivar, and 1470 to 1782 in the HA-259 cultivar in the presence of the pathogen. The lowest number of seeds per head (711) was recorded in 17577 cultivar when sprayed with jasmonic acid, a result differing significantly from that in B-224 (1034) and HA-259 (1163 at low concentration (2 mM) in the presence of *M. phaseolina*). Without any seed and foliar application, the total number of seeds fell to 775 in the 17577 cultivar, 868 in B-224, and 981 in HA-224 (Fig. 2). A similar trend was recorded during 2018 when the number of achene per head increased with maximum concentration.

Test weight (100-seed weight)

The analysis of variance showed that the main effects regarding test weight were significant (Table 2), except the seasons. The 100-seed weight tended to increase with both priming methods, with significantly different behaviour in the presence of charcoal rot. Abscisic acid seed treatment delivered the maximum 100-seed weight in HA-259 (9.06 g) followed by SA (8.48 g), GA (7.53 g) and JA (6.27 g) at higher concentration (Fig. 3). On the other hand, in 17577 the minimum 100-seed weight (3.55 g) was observed with JA at 2 mM concentration. A similar trend was recorded in the foliar application, with a maximum 100-seed weight (7.85 g) recorded in HA-259, followed by B-224 (6.74 g) and 17577 (6.55 g) at higher concentration.

Plant height (cm)

Significantly different plant heights were observed with seed and foliar application in the presence of *M. phaseolina.* The analysis of variance demonstrated that all the main effects regarding plant height were significant (Table 3). An increase in plant height was recorded with AA seed treatment in three sunflower genotypes at a higher concentration; the maximum (192.1 cm) was observed in HA-259, followed by B-224 (153.2 cm) and 17577 (112 cm). The same trend was found with foliar application of AA at a higher concentration in HA-259 (174 cm), B-224 (139 cm), and 17577 (103 cm). Plant height was slightly reduced with the application of 4 mM and 2 mM concentration of each priming agent with both application methods (seed and foliar) as compared to 6mM (Fig. 4). In SA application the height of plants was maximum at high concentration (6 mM) as compared to 4 mM, 2 mM, and control in B-224 germplasm: 146.2 cm, 118 cm, 111.2 cm, and 88 cm respectively. A similar trend was recorded in susceptible germplasm (17577), where AA exhibited the maximum height followed by SA, GA, JA, and control. Plant height did not exceed 96.9 cm with JA at 2 mM concentration with seed application. The results revealed that plant height also depends on the dose. The maximum dose increased the maximum plant height, and foliar application resulted in less plant height compared to seed treatment.

Table 3
Analysis of variance for main effects and their associated interactions for plant height, stem girth and shoot weight of sunflower in an experiment with two application methods, two seasons, three germplasm, four priming agents and four concentrations (total df = 575).

		F values[a]		
Source of variation	df	Plant height (cm)	Stem girth (cm)	Shoot weight (g)
Application	1	635.31**	274.75**	1246.25**
Season	1	7.50*	0.96[ns]	0.97[ns]
Germplasm	2	2679.45**	4407.66**	4952.77**
Priming agent	3	308.16**	189.67**	4523.07**
Concentration	3	2228.31**	6436.47**	6573.20**

[a]Levels of probability: ns, not significant; **, 5%

Stem girth (cm)

The main effects regarding stem girth (cm) were significant, except for the seasons (Table 3). Maximum stem girth was observed in HA-259, followed by B-224 and 17577, in both seasons. HA-259 exhibited the maximum 10.5 cm stem girth with abscisic acid seed treatment at 6 mM concentration; girth was 9.8 cm with the foliar application and 5.6 cm in control. B-224 exhibited 9.4 cm with seed treatment and 9.1 cm and 5.1 cm in foliar and control, respectively. The minimum 4.3 cm stem girth was observed in 17577 in control, while foliar application resulted in 6.5 cm (Fig. 5) and seed application resulted in 7.1 cm with abscisic acid treatment at 6 mM concentration. A similar trend was observed in SA, GA, and JA, followed by AA.

Shoot weight (g)

There was a significant difference in the main effects upon shoot weight, except the seasons (Table 3). Shoot weight of susceptible (17577) and moderately resistant germplasm (B-224 and HA-259) showed an increasing trend, being most evident with seed application of AA at 6mM concentration as compared to other priming agents (SA, GA, and JA) and application method (foliar application) in the presence of *M. phaseolina*. The maximum shoot weight was recorded in HA-259 (1216.1 g) with AA seed treatment at a higher concentration. Shoot weight also increased with SA application (1151.7 g), followed by GA (936.3 g) and JA (846.8 g) at the same concentration (Fig. 6). The minimum shoot weight was observed in 17577 (562.9 g) at 2mM concentration with foliar application of JA. Seed treatment delivered a better result than foliar application.

Root wet weight (g)

The analysis of variance showed that all the main effects upon root wet weight were significant, except seasons (Table 4). Our findings confirmed that the application of priming agents, be it to seed or foliar, has a positive impact on root wet weight. Seed treatment significantly increased root wet weight as compared to foliar application. Reduction in root wet weight (311g) was observed in 17577 with JA at 2 mM in seed priming while 103.9 g in foliar application. Among all priming agents, abscisic acid in both application methods delivered the maximum root wet weight (806.5 g) in HA-259 at maximum concentration (Fig. 7), followed by SA (759.5 g), GA (739.9g) and JA (726.2g) in seed treatments. The reduction in root wet weight of HA-259, B-224, and 17577 in control was recorded as 435.8 g and 308.1 g and 125.9 g, respectively.

Table 4
Analysis of variance for main effects and their associated interactions for root wet weight, root dry weight, and chlorophyll content of sunflower in an experiment with two application methods, two seasons, three germplasm, four priming agents, and four concentrations (total df = 575).

		***F* values**[a]		
Source of variation	df	Root wet weight (g)	Root dry weight (g)	Chlorophyll content (mg/g)
Application	1	1742.50**	320.82**	416.60**
Season	1	0.16[ns]	4.84*	5.94*
Germplasm	2	36426.2**	49491.0**	10956.3**
Priming agent	3	478.32**	2756.63**	671.30**
Concentration	3	6244.84**	14848.1**	3647.45**

[a]Levels of probability: ns, not significant; **

Root dry weight (g)

The analysis of variance confirmed that all the main effects of root dry weight (g) were significant (Table 4). Root dry weight (g) was significantly higher (746g) in HA-259 with AA seed treatment at higher concentration, followed by B-224 (542.9 g) and 17577 (353.3 g) in the presence of charcoal rot. Minimum dry weight (103.4g) was observed in 17577 with JA at 2 mM concentration. A significant difference was noted in foliar application with AA at higher concentration among germplasm HA-259 (723.9 cm), B-224 (524 cm), and 17577 (340 cm), respectively (Fig. 8). AA delivered the best results at maximum concentration, followed by SA, GA and JA.

Chlorophyll content (mg/g)

The analysis of variance showed that all the main effects of chlorophyll content were significant (Table 4). Chlorophyll content observed in plants of susceptible (17577) and moderately resistant germplasm (B-224 and HA-259) showed an increasing trend, this being most evident with AA seed application at 6mM concentration as compared to other priming agents (SA, GA, and JA), the application method (foliar application) and control in the presence of *M. phaseolina*. Maximum chlorophyll content (0.74 mg/g) was recorded in HA-259 with AA seed treatment at 6mM concentration. JA also resulted in increased chlorophyll content (0.42 mg/g), but that was not significantly high; this was followed by of GA (0.54 mg/g) and SA (0.68 mg/g) at the same concentration (Fig. 9). Similarly, maximum chlorophyll content was observed in HA-259 (0.67 mg/g) with foliar application of AA at a high concentration (6 Mm), while JA also delivered an increased content (0.35 mg/g) but not at a significantly high level, followed by SA (0.62 mg/g) and GA (0.47 mg/g) at the same concentration, while in the case of non-primed plants 0.25 mg/g chlorophyll content was recorded.

Discussion

Both seed and foliar applications of priming agents have been found to enhance germination rate, uniformity, and metabolic activities, while also triggering defense responses against biotic and abiotic stresses, with positive impacts on physiological changes in plants (Raj and Raj, 2019). They have also been found to improve growth parameters in various plant species such as *Daucus carota*, *Matricaria chamomilla* (Kováčik et al., 2009), *Aloe vera* (Miri et al., 2014), *Solanum lycoperscium* (Patel and Rai, 2018), and *Solanum melongena* (Ali et al., 2019).

In the present research, a reduction in the number of seeds per head was observed in susceptible germplasm under the biotic stress of charcoal rot disease. The head diameter of the sunflower contributes as much as 55.56% toward sunflower seed yield and varied from 20 cm to 30 cm. Our results are in line with Skoric (2012), who found that the number of seeds per head was reduced in sunflower crops under different biotic and abiotic stresses, resulting in reduced sunflower yields. The range of head diameter was 20.33–31.63 cm in HA-259 with seed and foliar application of four priming agents (SA, GA, JA, AA), which showed great potential to enhance the yield. These findings match those of Rafi et al. (2015), who reported that the application of priming increased the shoot weight, root fresh and dry weight of sunflower (*Helianthus annuus*), chickpea *(Cicer arietinum)*, okra (*Abelmoschus esculentu*), and peanut *(Arachis hypogaea*) as compared to unprimed plants. Our results about head diameter, achene weight, and the number of achene per head were also in line with the findings of Asghar et al. (2019), who confirmed that seed priming has a significant impact on the yield, head diameter, achene weight and achene per head of two sunflower genotypes (Hysun-33 and FH-331) as compared to non-primed control. A study using *Pseudomonas fluorescens* as seed priming against abiotic stress also observed physiological parameters that increased dramatically in primed plants compared to control (Pravisya et al., 2019).

The present study found that sunflower height increased significantly with seed and foliar application of priming agents at three concentrations in the presence of charcoal rot as compared to unprimed plants. Similar results were obtained by Shah et al. (2018) regarding seed priming with GA, which enhanced seed germination, plant height, stem length, and stem thickness of Lisianthus plants (*Eustoma grandiflorum*). Seed treatment with gibberellic acid also enhanced plant height in chickpea (Shariatmadari et al., 2017) under drought stress in glasshouse and field conditions. The results are confirmed by Zayan (2016), who primed the seed of okra plants with salicylic acid (50, 100, and 200 ppm), ascorbic acid (50, 100, and 200 ppm), Bion (200, 400, and 800 ppm) and humic acid (1000, 2000 and 4000 ppm) and observed the impact of priming agents on plant height and yield under charcoal rot stress in two consecutive years (2013–2014). The increase in physiological parameters and reduction in disease severity of charcoal rot found with increasing the concentration in primed plants as compared to unprimed is in line with the present findings. The same trend was observed by Noreen et al. (2017) with regard to the impact of salicylic acid on chlorophyll content, 100-grain weight, total grain weight, root and shoot tissue in wheat varieties under water stress and the improvement in all these parameters with a high dose of SA as compared to low and control. The findings of (Zayan, 2016) with regard to seed treatment with priming agents of *Abelmoschus esculentu*s with wheat plants were similar to the current findings with sunflower plants, as the increase in physiological parameters was recorded at a maximum dose of each priming agent in both seed treatment and foliar application as compared to untreated plants. Similarly, Kalaivani et al. (2016) treated the seed of rice varieties with different concentrations (75, 50, 25, and 100 mg/L) of methyl salicylate (MeSA) to explore the effect on the physiology of plants and observed that seedling emergence was enhanced at a higher concentration when compared to lowers. Additionally, MeSA not only modified plant physiology but was also useful for crop production and protection. The results are further supported by the findings of Karthika and Vanangamudi (2013), who reported that maximum concentration of seed bio priming (phosphobacteria and Azospirillum) led to increased physiological parameters (dry matter, root shoot length) of maize compared to control.

Our present results are in line with the findings of Wahid et al. (2008), who found that the application of ascorbic acid, gibberellic acid, and salicylic acid has positive impacts on physiological parameters such as shoot and root length and the shoot and root dry weight of sunflower. Similarly, Noreen and Ashraf (2008) applied three doses of salicylic acid as a foliar application on two sunflower lines (Hisun-33 and SF-187) under abiotic stress and found an appreciable increase in root and shoot fresh and dry weight as compared to untreated plants.. Similarly, Dai et al. (2017) compared seed priming and hydro-priming with different chemicals including gibberellic acid to investigate the effect on shoot weight, root weight, and chlorophyll content in *Glycine max* seedlings. They found more increase in chlorophyll in seed priming as compared with hydropriming. Our results are also in line with those of Khan et al. (2018), who studied the effects of zinc and farm-yard manure at different concentrations (1.25, 2.44 and 5 mg kg^{-1} and 1% and 2%) on chlorophyll content, grain yield, 100-seed weight, shoot fresh/dry length in *Vigna radiata* in the presence of *M. phaseolina* and found that physiological parameters increased at maximum concentration.

The present study demonstrated that seed priming with AA improves physiological parameters, including chlorophyll content of sunflower, as compared to GA and other priming agents. These findings are supported by those of Farahmandfar et al. (2013), who compared the effects of sodium chloride, SA, GA and hydro-priming on chlorophyll content, dry weight, and length of plumule and seedling radicle of *Trigonella foenum graecum*. They found that seed treatment with salicylic acid enhanced the physiological parameters of fenugreek seedlings more effectively than gibberellic acid and other treatments. Our results are also in line with those of Razzaq et al. (2013), who studied the effects of seed priming with salicylic acid (50, 100, 150 ppm), abscisic acid (50, 100, 150 ppm) and ascorbic acid (10, 20, 30 ppm) on membrane stability index, chlorophyll content, relative water content (RWC) root-shoot ratio and yield in wheat cultivars, and found that salicylic acid delivered pronounced and better effects than other chemicals. Similarly, El-Hai et al. (2009) investigated the effect of exogenous and endogenous application of citric acid and salicylic acid against charcoal rot, observing various physiological parameters of sunflower (chlorophyll content, stem diameter, flower head diameter, seed plant yield, and 100-seed weight). All treatments with antioxidants reduced the incidence of charcoal rot disease, and physiological parameters were significantly increased in sunflower crops under field conditions. The studies undertaken by Youssef et al. (2017) found that exogenous application on sunflower plants with SA (0.7 and 1.4mM) played a vital role in increasing yield, head diameter, plant height, total seed per head, and chlorophyll content (a,b), while also mitigating abiotic stress at higher concentration. A similar trend was found in the present study, with our results indicating that physiological parameters are enhanced with the application of priming agents at a higher concentration. Exogenous application (acibenzolar-S-methyl and exopolysaccharides) has been shown to improve physiological properties and stimulate the defense mechanism in tomato against bacterial spots (Blainski et al., 2018).

Presents results were also strengthen by the findings of (Kalpana et al., 2015) regarding the agronomical parameters. They reported that plant height, dry weight, and chlorophyll content increased in wheat plants primed with KCl, KNO_3, and GA against biotic stress, and among them GA was found superior to other priming agents. Similar findings were reported by Arun et al. (2017), who investigated the effects of seed priming with $CaCl_2$, GA, $ZnSO_4$, and MG $(NO_3)_2$ on different physiological parameters in cowpea cultivars. The results indicated that seed treatment with GA enhanced all physiological parameters more efficiently as compared to other priming agents. Langeroodi and Noora (2017) reported a significant increase in physiological parameters (chlorophyll content, relative water content, and seed yield) by applying gibberellic acid as seed treatment in soybean under water stress. An increment was noted in physiological parameters (root shoot length, dry matter, relative water content) by foliar application of SA in maize against TMV in comparison to control (AA et al., 2019).

Conclusion

Priming technique is used not only to increase the crop yield of crops worldwide but also enhances the level of tolerance against biotic and abiotic stresses. The present study clearly showed that seed treatment with priming agents, specifically SA,AA, GA and JA, is an environmentally sound, easy to apply, and more reliable approach to manage charcoal rot disease of sunflower as compared to foliar application. Abscisic acid has the most positive impact on plant physiological parameters (head diameter, 100-seed weight, number of achene per head, plant height, stem girth, shoot fresh and dry weight, root fresh and dry weight, and chlorophyll content), followed by salicylic acid, gibberellic acid, and jasmonic acid, in that order. Seed priming can be a valuable element of integrated disease management of charcoal rot of sunflower for its sustainable production.

Declarations

Acknowledgement: The authors are highly indebted to Judith Riedel Director Agroecology.Science Ltd, Institute of Sustainable Food and Farming Systems Frick, Switzerland for critically reviewing and improving the English.

Funding (information that explains whether and by whom the research was supported)

No funding was received to assist with the preparation of this manuscript and to conducting this study. The authors further certified that no financial interests are involved. The authors have no relevant financial or non-financial interests to disclose

Conflicts of interest/Competing interests (include appropriate disclosures)

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval (include appropriate approvals or waivers)

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript. Authors are responsible for correctness of the statements provided in the manuscript. This is an observational study no ethical approval is required

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All the authors agreed that datasets on which the conclusions of the paper relied is available to readers

Code availability (software application or custom code)

The authors used the software obtained by the Institute. We understand that the text and any pictures or videos published in the article

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

MUG, MIQ conceive the idea, AH and MFA collected materials, coordinate, and writing of the manuscript. All authors have approved the final version of the manuscript.

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Figures

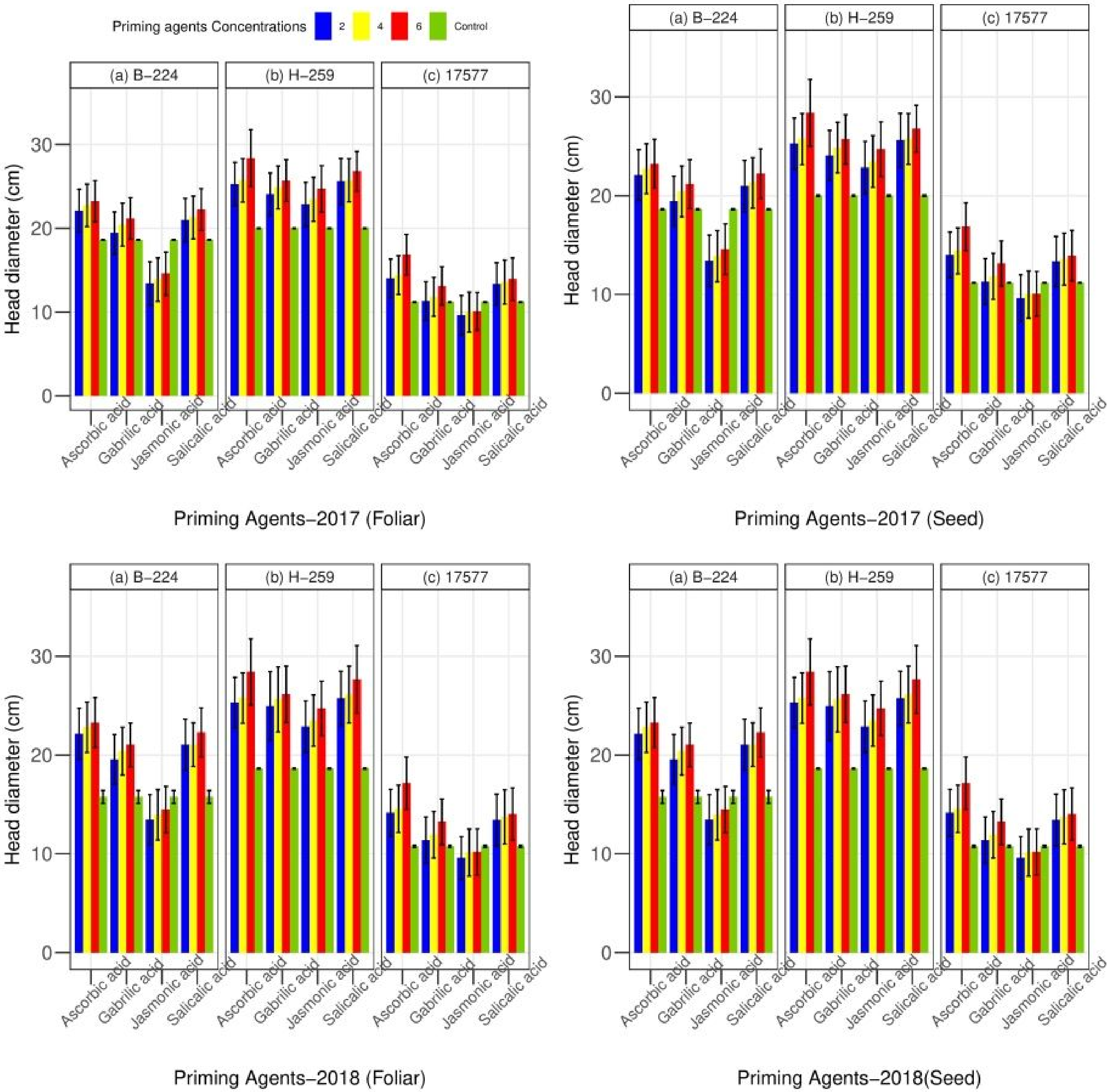

Figure 1

Mean comparison of main effects of application methods (seed and foliar), seasons (2017, 2018), germplasm (B-224, HA-259 and 17577), priming agents (SA, GA, JA, and AA), and concentrations (2, 4 and 6 mM and control) on head diameter (cm) of the sunflower. Data are means of two repeated experiments. All means for the same main effect followed by a different letter are significantly different based on Tukey's range test (0.05).

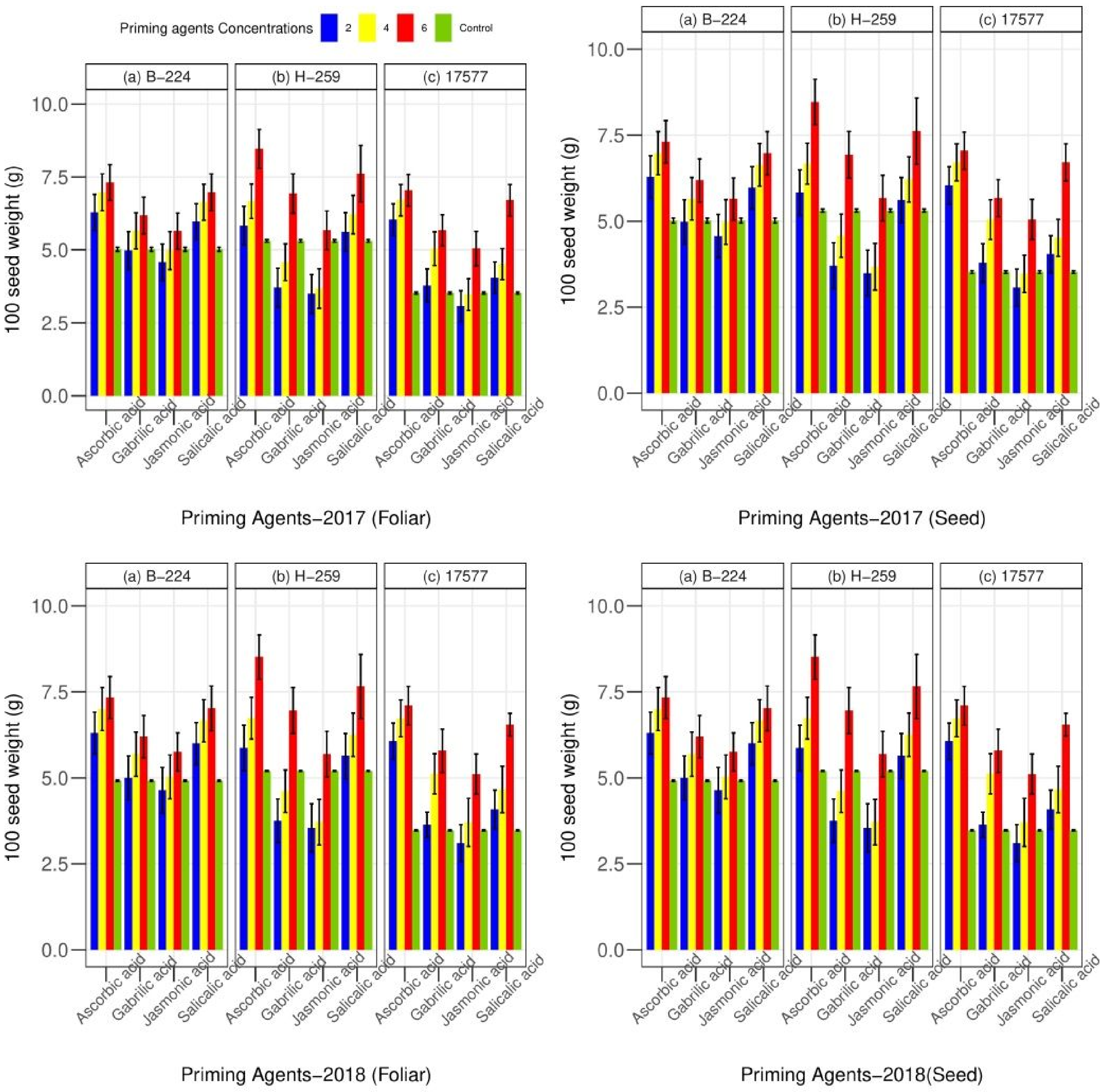

Figure 2

Mean comparison of main effects of application methods (seed and foliar), seasons (2017, 2018), germplasm (B-224, HA-259 and 17577), priming agents (SA, GA, JA, and AA), and concentrations (2, 4 and 6 mM and control) on 100-seed weight (g) of the sunflower. Data are means of two repeated experiments. All means for the same main effect followed by a different letter are significantly different based on Tukey’s range test (0.05).

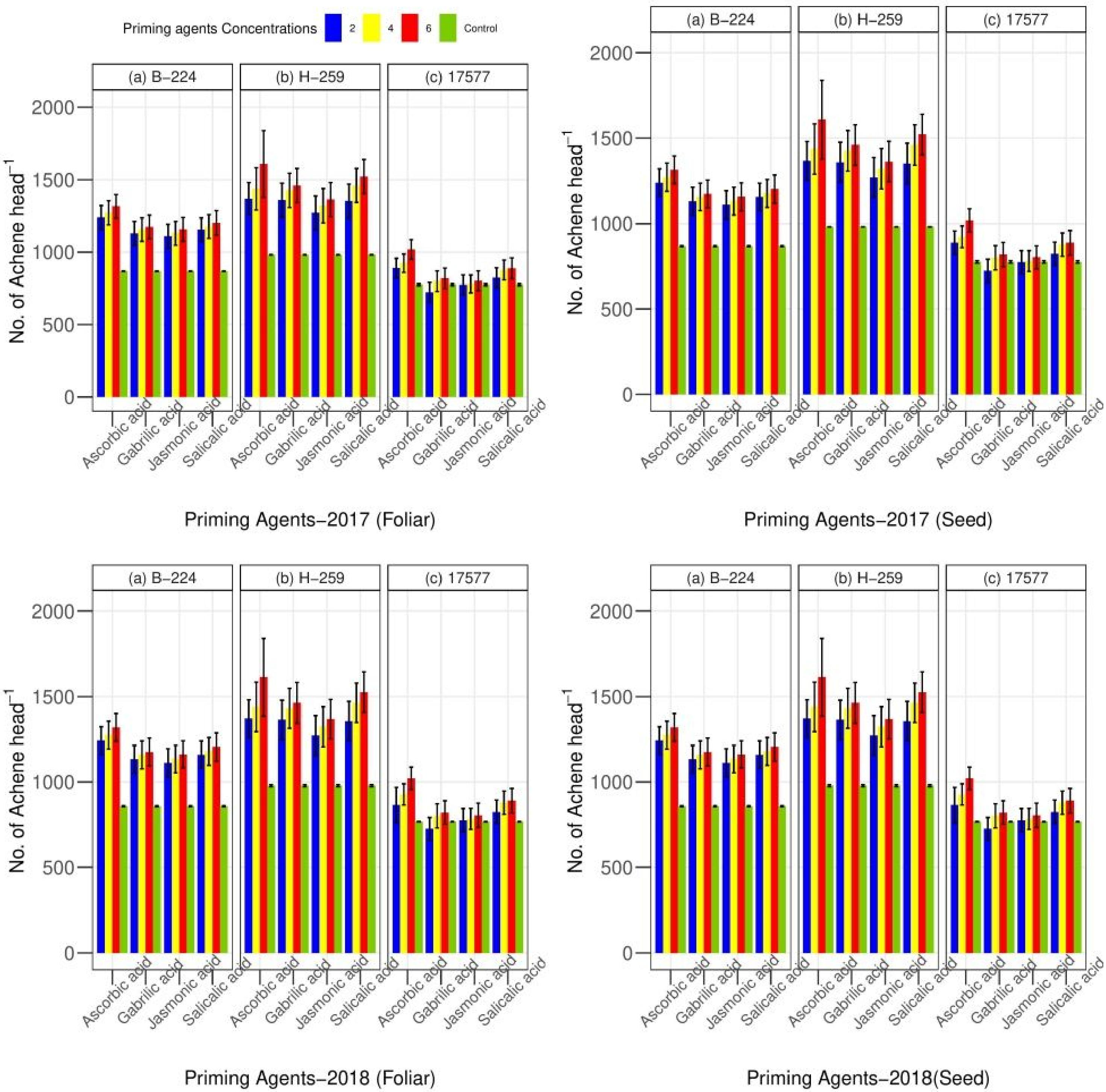

Figure 3

Mean comparison of main effects of application methods (seed and foliar), seasons (2017, 2018), germplasm (B-224, HA-259 and 17577), priming agents (SA, GA, JA, and AA), and concentrations (2, 4 and 6 mM and control) on no. of achene per head on the sunflower. Data are means of two repeated experiments. All means for the same main effect followed by a different letter are significantly different based on Tukey's range test (0.05).

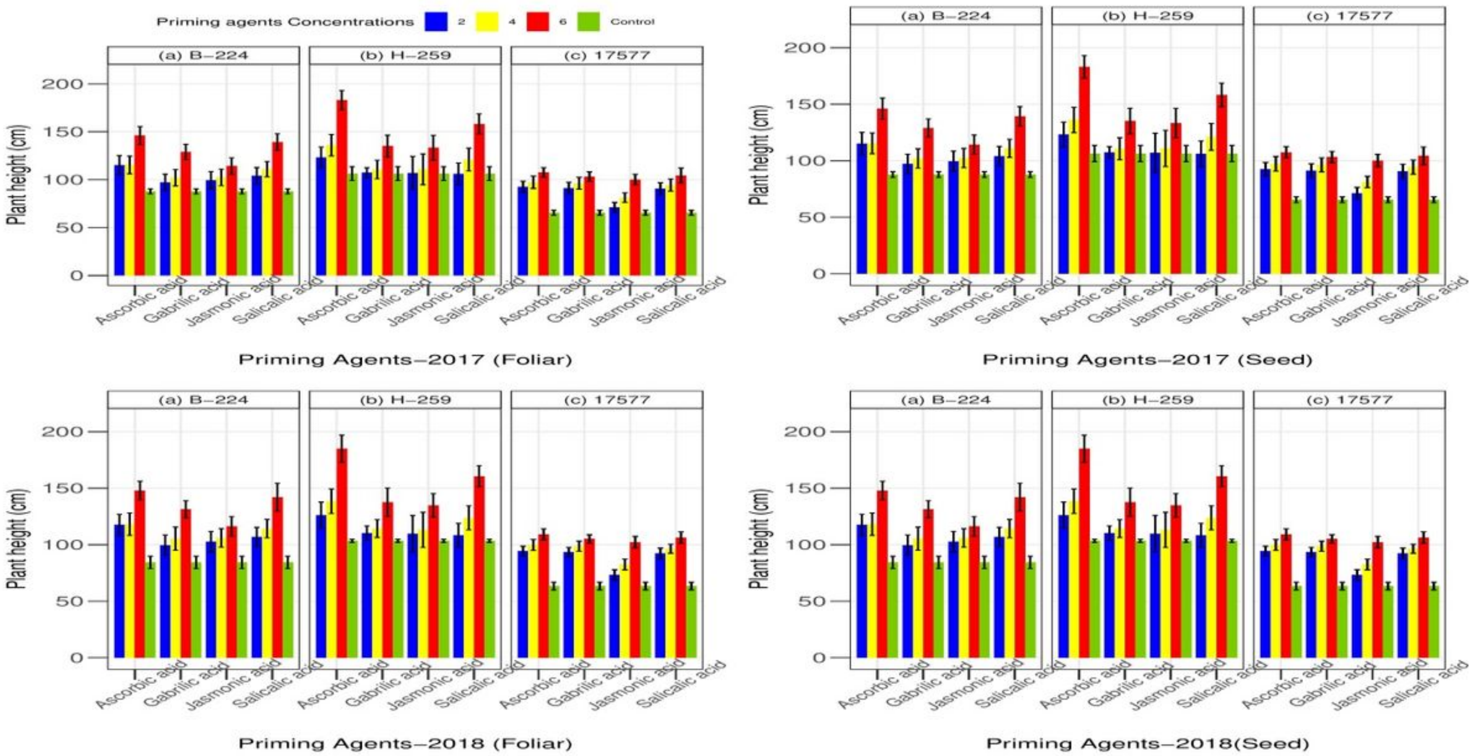

Figure 4

Mean comparison of main effects of application methods (seed and foliar), seasons (2017, 2018), germplasm (B-224, HA-259 and 17577), priming agents (SA, GA, JA, and AA), and concentrations (2, 4 and 6 mM and control) on plant height (cm) on the sunflower. Data are means of two repeated experiments. All means for the same main effect followed by a different letter are significantly different based on Tukey’s range test (0.05).

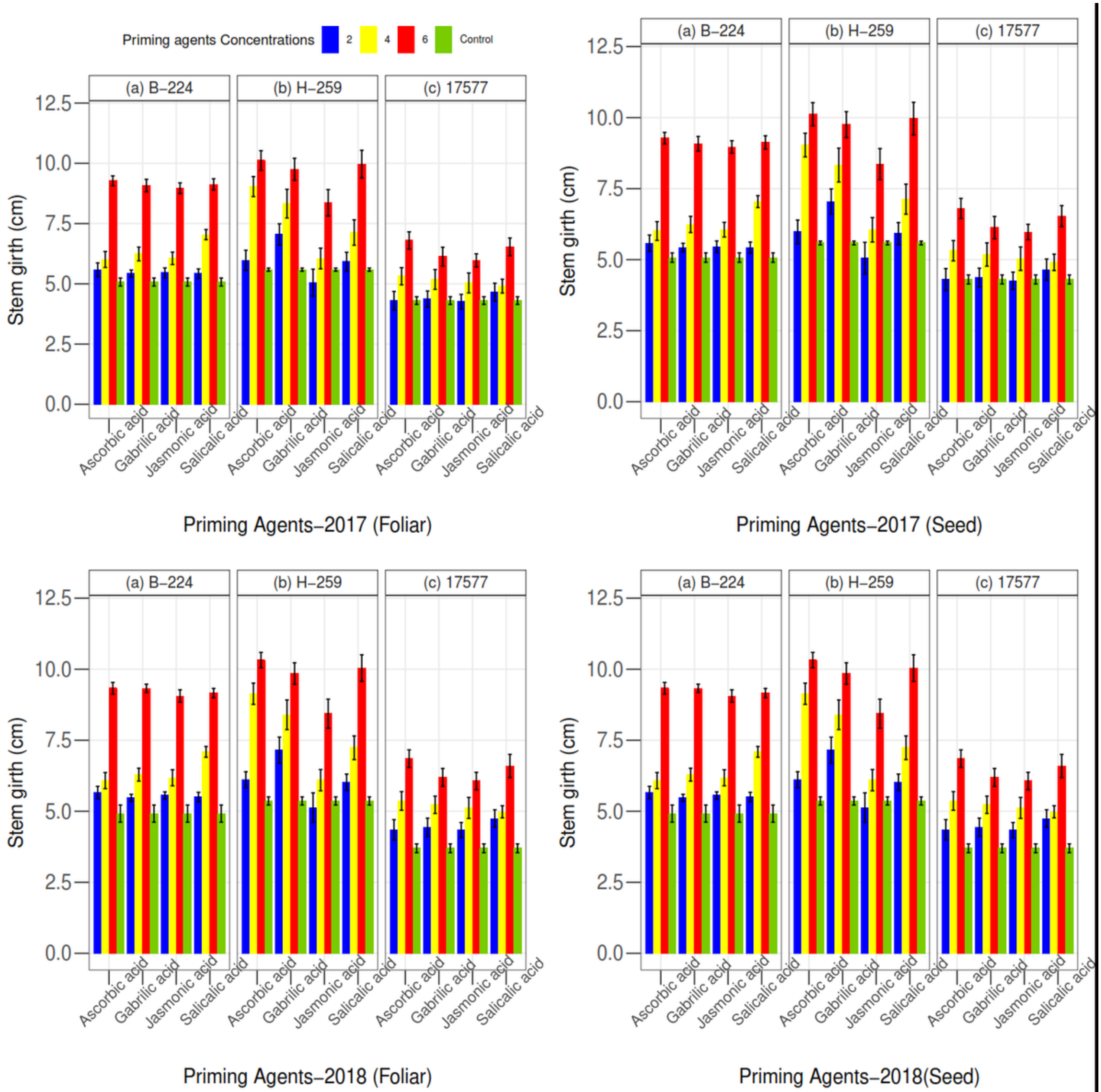

Figure 5

Mean comparison of main effects of application methods (seed and foliar), seasons (2017, 2018), germplasm (B-224, HA-259 and 17577), priming agents (SA, GA, JA, and AA), and concentrations (2, 4 and 6 mM and control) on stem girth (cm) on the sunflower. Data are means of two repeated experiments. All means for the same main effect followed by a different letter are significantly different based on Tukey's range test (0.05).

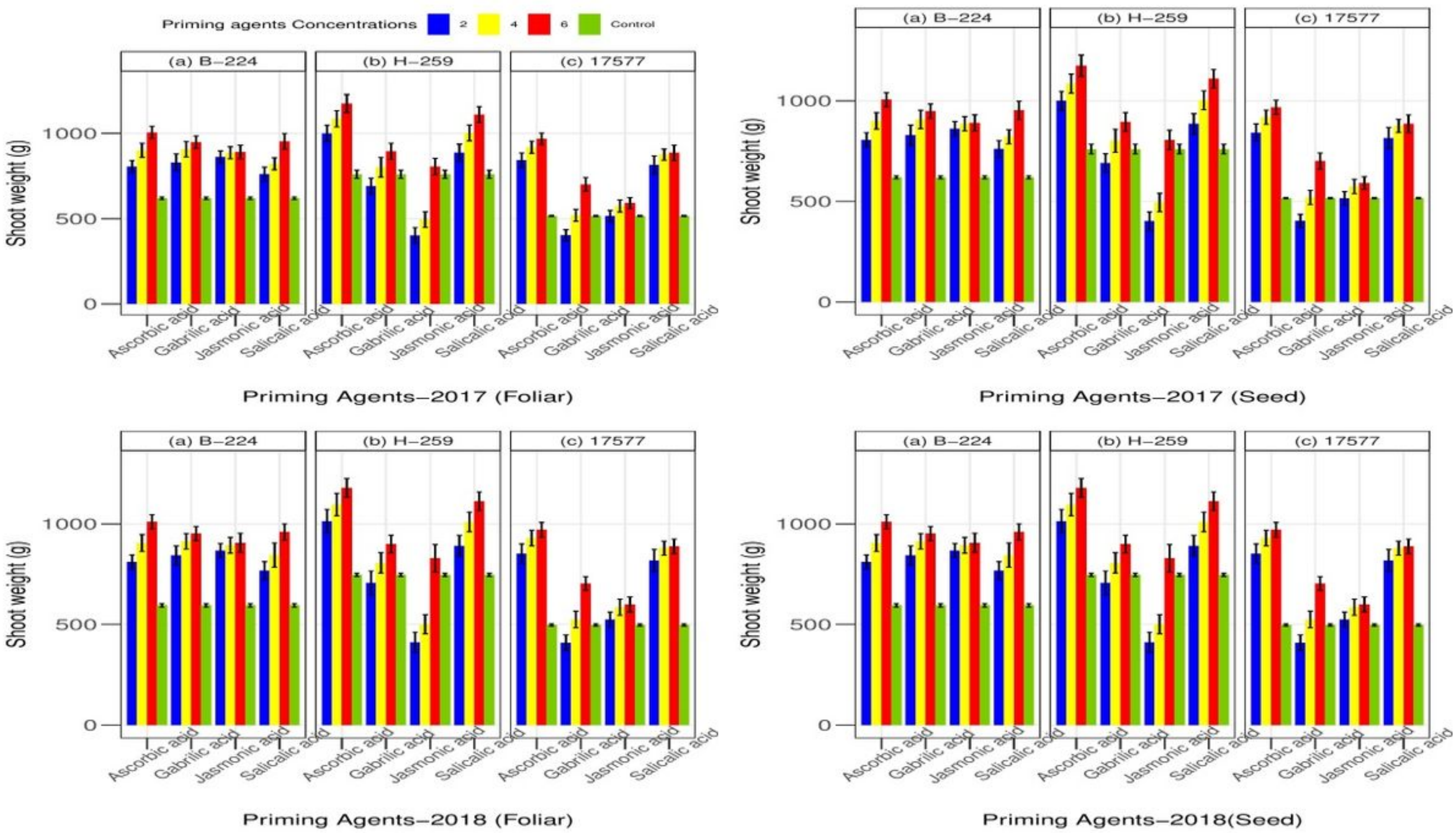

Figure 6

Mean comparison of main effects of application methods (seed and foliar), seasons (2017, 2018), germplasm (B-224, HA-259 and 17577), priming agents (SA, GA, JA, and AA), and concentrations (2, 4 and 6 mM and control) on shoot weight (g) on the sunflower. Data are means of two repeated experiments. All means for the same main effect followed by a different letter are significantly different based on Tukey's range test (0.05).

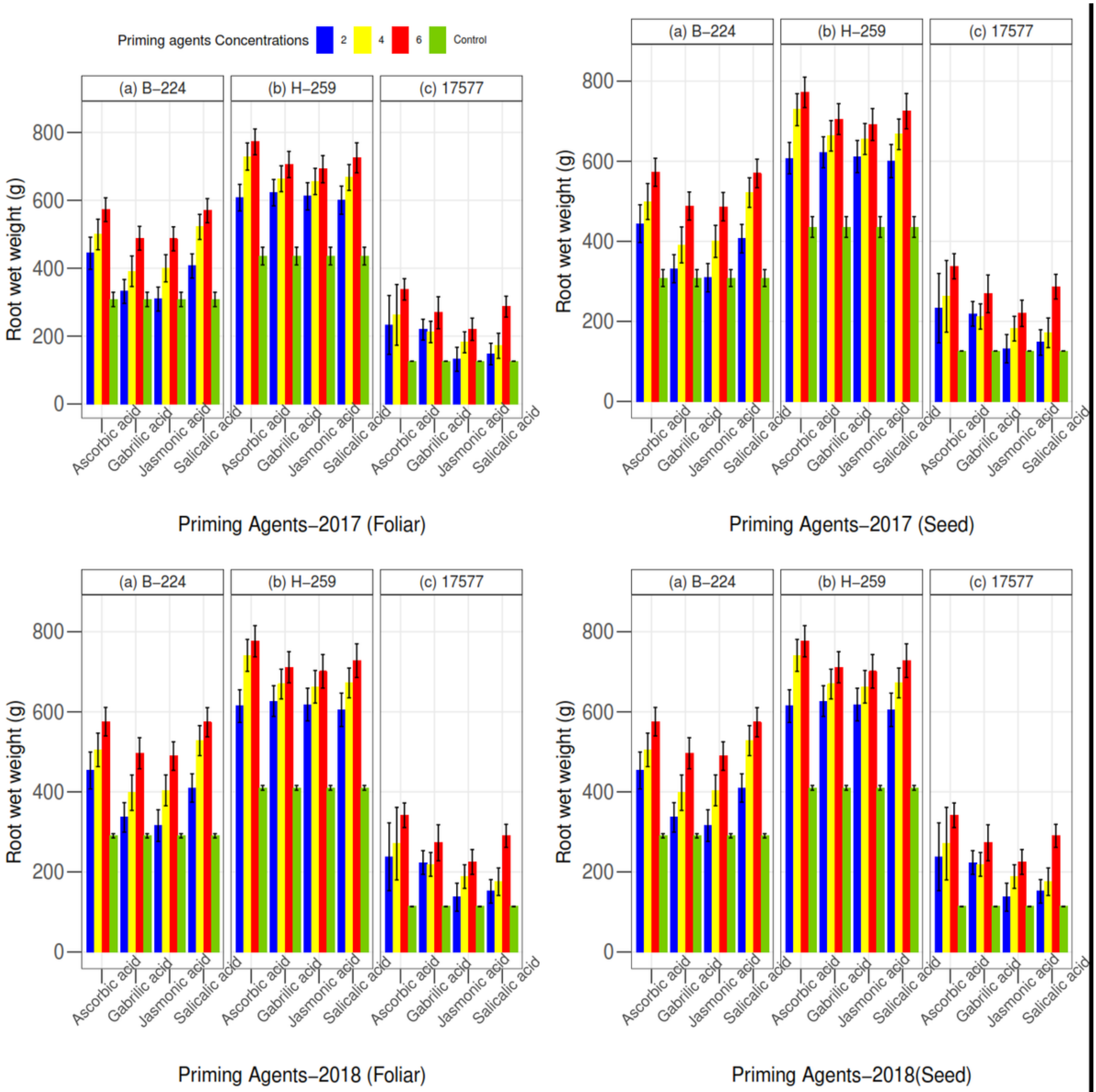

Figure 7

Mean comparison of main effects of application methods (seed and foliar), seasons (2017, 2018), germplasm (B-224, HA-259 and 17577), priming agents (SA, GA, JA, and AA), and concentrations (2, 4 and 6 mM and control) on root wet weight (g) on the sunflower. Data are means of two repeated experiments. All means for the same main effect followed by a different letter are significantly different based on Tukey’s range test (0.05).

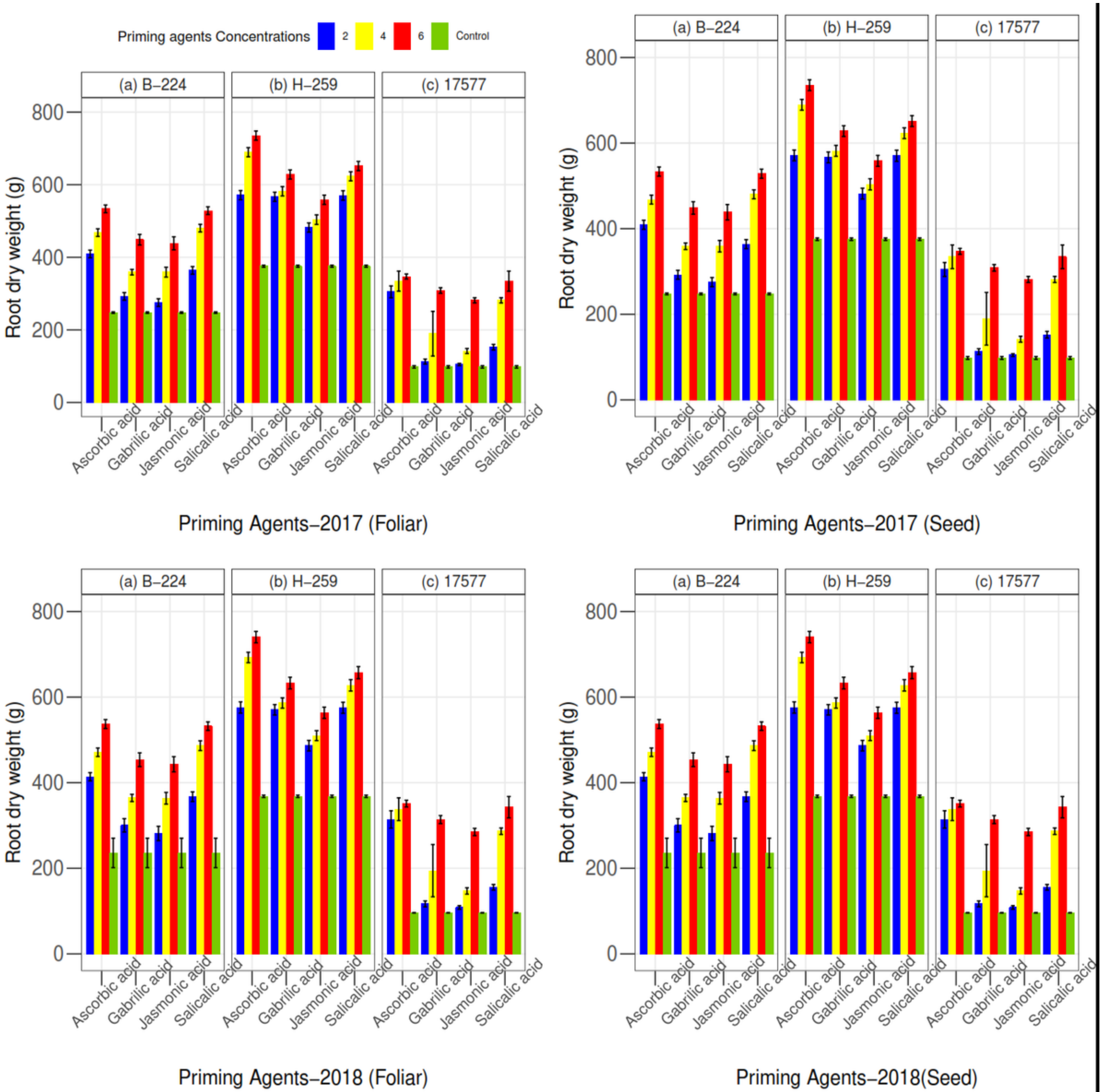

Figure 8

Mean comparison of main effects of application methods (seed and foliar), seasons (2017, 2018), germplasm (B-224, HA-259 and 17577), priming agents (SA, GA, JA, and AA), and concentrations (2, 4 and 6 mM and control) on root dry (g) on the sunflower. Data are means of two repeated experiments. All means for the same main effect followed by a different letter are significantly different based on Tukey's range test (0.05).

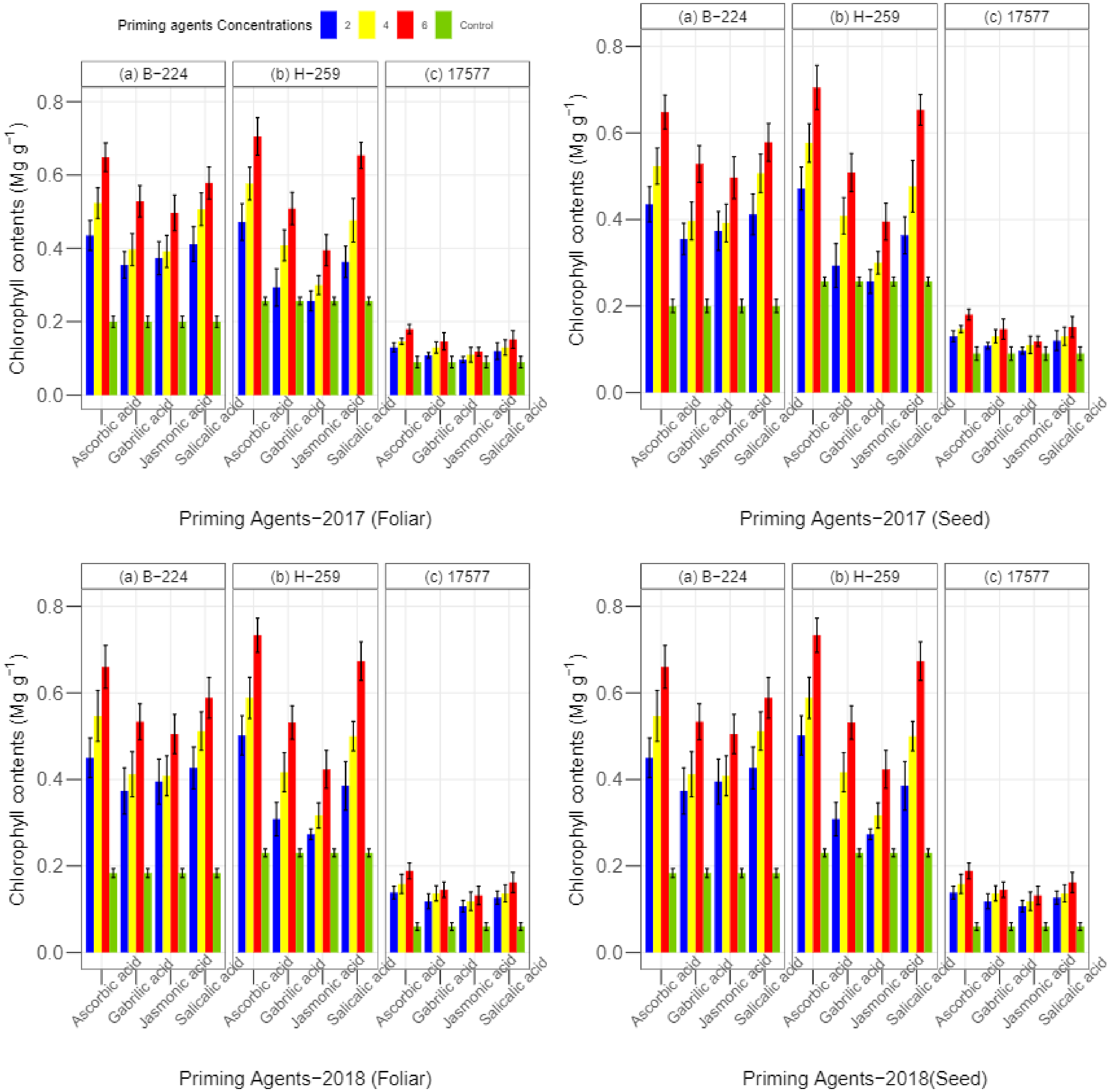

Figure 9

Mean comparison of main effects of application methods (seed and foliar), seasons (2017, 2018), germplasm (B-224, HA-259 and 17577), priming agents (SA, GA, JA, and AA) and concentrations (2, 4 and 6 mM and control) on chlorophyll content (mg/g) of the sunflower. Data are means of two repeated experiments. All means for the same main effect followed by a different letter are significantly different based on Tukey’s range test (0.05).