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REGULAR ARTICLE

Potassium application alleviates grain sterility and increases yield of wheat (*Triticum aestivum*) in frost-prone Mediterranean-type climate

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Abstract
Aims Frost is a major risk factor for grain production in Australian farming systems and appears to be increasing in severity and occurrence due to changing climate. In this study we assessed the role of potassium (K) and micronutrients in alleviating floret sterility (FS) and yield loss in wheat crops subject to frost.
Methods Field experiments with K application in 2015 and 2016 were conducted in frost-prone, low soil K fields in the grain belt of Western Australia. Following frost events the heads reaching anther dehiscence were tagged and FS was measured 5–6 weeks later. We also measured leaf K concentration, photosynthesis and antioxidant activity, and grain yield.
Results In 2015 K supply decreased FS from 32% at nil K to 24% at 80 kg K ha^{-1}. In 2016 the FS values varied from 30 to >95%. Although there was no effect of K on FS at extreme frosts (FS >95%), applying 20–80 kg K ha^{-1} reduced FS by 10–20% and increased yield by 0.2–0.4 t ha^{-1} at less severe frosts. The decrease in FS was associated with increasing leaf K concentrations in the range 1.5–2.6%, higher photosynthesis and less oxidative stress at anthesis, but K supply did not provide extra protection from frost damage at leaf K > 2.6%. Foliar micronutrients at booting and heading did not affect FS in either year due to adequate micronutrient levels in the topsoil.
Conclusions Improved plant K status can increase grain set and yields in wheat under frost, likely by maintaining physiological functions such as cell osmoregulation, plant photosynthesis and anti-oxidant systems. Plant K requirement in frost prone parts of the landscape is greater than in the areas with low risk of frost damage.

Keywords Wheat · Grain yield · Potassium nutrition · Frost damage · Floret sterility

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Introduction

Although Australia's climate is warming, the number of frost days and the length of the frost season have increased across much of the Australian grain belt because the southerly shift of high pressure systems during winter and early spring brings more cold air masses from over the Antarctic than in the past (Crimp and Christopher 2014). Following cold days, frosts often occur when nights are clear and calm as a result of radiative heat loss from the ground and the crop itself. Cereal crops are most susceptible to frost damage during and after flowering, due to the sensitivity of pollen development at the young microspore stage to frost (Parish et al. 2012; GRDC Grownotes 2016). For example, the 2016 season delivered the coldest September since records began in Western Australia, resulting in a series of frost events that severely damaged wheat and barley crops particularly in the central grain belt with an estimate of grain losses of at least 1.5 million tonnes (Braidotti 2017).

There are two types of cold stress, chilling and freezing, known to damage cereal crops (Rebbeck and Knell 2007). Chilling injury often occurs when plants are exposed to temperatures of <10 to 0 °C and mostly relates to metabolic dysfunctions (e.g. disruption to membranes, metabolism or photosynthesis) that in turn causes sterility (Huang et al. 2005). In contrast, freezing injury occurs at temperatures lower than −2 °C when ice forms in plant tissues, causing either mechanical damage or cell dehydration (Pearce 2001). Both chilling and freezing result in photo-oxidative damage to chloroplasts as a consequence of high light energy absorbance in excess of the capacity of chloroplasts to use it for CO_2 fixation at low temperature. This excess energy would be used for the formation of reactive oxygen species (ROS) (Huner et al. 1998; Foyer et al. 2002), that are highly toxic, causing membrane damage and chlorophyll degradation, and are thus responsible for development of leaf chlorosis and necrosis following frost events (Cakmak 2005).

The role of potassium (K) in protecting crops against frost damage has been suggested for many years and discussed in plant nutrition textbooks (Bergmann 1992; Marschner 1995). Alleviation of foliar frost damage by increasing K supply was demonstrated in a field experiment with potato grown on a number of alluvial soils with different K levels (Grewal and Singh 1980). Increasing K concentration in the irrigation water also protected stem damage from low night temperatures in carnation plants (Kant and Kafkafi 2002). Similarly, the resistance to low temperatures (4–16 °C) in tomato, pepper, and eggplant seedlings was enhanced by K application (Hakerlerler et al. 1997). However, little has been known about the effect of K nutrition on frost resistance in cereal crops, despite the anecdotal reports from agricultural consultants and growers in Western Australia that wheat crops are more susceptible to frost damage on low K soils.

In this study, we conducted field experiments at frost-prone, low soil K sites to determine whether high tissue K concentrations are associated with reduced frost susceptibility in wheat. The magnitude of reduced frost susceptibility by K supply was evaluated in terms of floret sterility (FS) presumably caused by frost by tagging the ears of flowering wheat plants immediately after a frost event and then counting the number of damaged grains out of the total florets (Biddulph et al. 2015). Flag leaf K concentrations, net photosynthesis, antioxidative activity, plant growth and grain yield were measured. While frost occurs in most years in the study area, the timing and severity varies. To maximise the coincidence of a frost event or events with critical phases of pollen development and anthesis, wheat was sown at 2-week intervals from mid-April to the end of May in each year.

Materials and methods

The 2015 experiment

Wheat (*Triticum aestivum* L.) cvs. Mace (moderately susceptible to frost; Biddulph et al. 2015) and Wyalkatchem (highly susceptible to frost; Biddulph et al. 2015) were grown on a farm at Aldersyde in the central grain belt, Western Australia (32°22’S, 117°17′ E). The soil was grey gravelly sand, and the crop in 2014 was canola. A pre-sowing soil analysis showed 48 mg K kg^{-1} (bicarbonate extraction, Colwell-K; Colwell and Esdaile 1968) at 0–10 cm, 29 mg K kg^{-1} at 10–20 cm and 34 mg K kg^{-1} at 20–30 cm (Table 1, see other soil properties). The experiment was sown at 80 kg of seeds ha^{-1}. Individual plots had a sown area of 1.65 m by 10 m and six rows at 0.25-m row spacing, trimmed to 1.65 m by 8 m at harvest. Basal fertilisers were 100 kg of urea ha^{-1} and 120 kg of diammonium phosphate ha^{-1} at sowing, plus 80 L ha^{-1} of a liquid fertiliser UAN (urea ammonium nitrate, 32% N) once post emergence.

Multiple sowings (15 April, 29 April, 15 May, and 2 June) were adopted to ensure that wheat would be flowering from early August to early October, the typical frost window for this region. Each sowing block was considered as a different environment. The first two sowings, before the break of the long dry season, were established with drip irrigation equivalent to 25 mm of rainfall for uniform crop emergence. Subsequent sowings emerged with soil moisture from rainfall. The rates of soil K supply using KCl were nil and 80 kg K ha^{-1} by broadcasting at sowing, plus a third treatment of 80 kg K ha^{-1} at sowing and foliar spray of micronutrients at booting

Table 1 Pre-sowing soil properties of the experimental sites at Aldersyde in 2015 and at Beverley in 2016

Site	Soil depth (cm)	Colwell K (mg kg^{-1})	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})	Colwell P (mg kg^{-1})	KCl-40 S (mg kg^{-1})	Org C (%)	EC (dS m^{-1})	pH_{CaCl2}	DTPA Cu) (mg kg^{-1})	DTPA Mn (mg kg^{-1})	DTPA Zn (mg/kg)	B_{CaCl2} (mg/kg)
Aldersyde	0–10	48	3	31	35	15	1.3	0.2	5.8	1.0	3.8	1.9	0.4
	10–20	29	2	3	33	6	0.4	0	4.2	0.4	0.8	0.2	0.4
	20–30	34	1	3	12	12	0.2	0	4.7	0.3	2.1	0.1	0.6
Beverley	0–10	42	10	33	40	22	1.4	0.2	5.3	0.5	10.7	0.9	0.4
	10–20	26	1	3	12	6	0.5	0	4.6	0.2	3.7	0.1	0.3
	20–30	25	1	2	3	4	0.2	0	5.5	0.2	1.6	0.1	0.2

and ear emergence. The foliar solution of micronutrients included 0.5% $CuSO_4$, 1% $MnSO_4$, 1% $ZnSO_4$ and 0.075% Na_2MoO_4 at a spraying rate of 80 L ha^{-1}. The factorial treatments of two cultivars and three nutrient rates were applied in a randomised complete block design with four replications for each of four sowing times, totalling 96 plots. To ensure more uniform effects of frost across the experimental site, it was sown as close to a continuous crop canopy as possible. Briefly, the interplot gap was reduced to a single row spacing to minimise plot edge effects and the pathways between plot ends were not removed until maturity. Buffer plots were also sown around the edges of the treatment plots to make a continuous canopy for each time of sowings.

The 2016 experiment

Wheat cv. Mace was grown near Beverley in the central grain belt of Western Australia (32°12’S, 116°45′E). The soil was sandy loam, and the crop in 2015 was oat. A pre-sowing soil analysis showed 41 mg K kg^{-1} (bicarbonate extraction) in the 0–10 cm layer, 26 mg K kg^{-1} at 10–20 cm and 25 mg K kg^{-1} at 20–30 cm (Table 1, see other soil properties). The experiment had the same seeding rate, plot size and row spacing as the 2015 experiment. Basal fertilisers were 100 kg of urea ha^{-1} and 100 kg of diammonium phosphate ha^{-1} at sowing, plus 80 L ha^{-1} of UAN (urea ammonium nitrate, 32% N) once post-emergence at about 30 kg N ha^{-1} in liquids.

The experiment was sown four times on 13 April, 4 May, 20 May and 10 June, respectively, to increase the probability of wheat at some sowing dates experiencing frost at flowering. Irrigation was not required for seedling emergence due to the early break of the season. The rates of soil K supply using KCl were nil, 20, 40, 80 kg K ha^{-1}, drilled below the seed. Additional treatments at nil, 80 kg K ha^{-1} were supplemented with foliar micronutrients or micronutrients plus K as 5% K_2SO_4 at booting and ear emergence. Foliar micronutrients included 0.5% H_3BO_3 and the same concentrations of Cu, Mn, Zn and Mo as that in 2015. The eight nutrient treatments at each of four sowings were replicated four times in a randomised complete block design, totalling 128 plots. As in 2015, the whole experiment was sown as close to a continuous crop as possible for uniform frost effect. Buffer plots were also provided around the treatment plots for each of the four sowings.

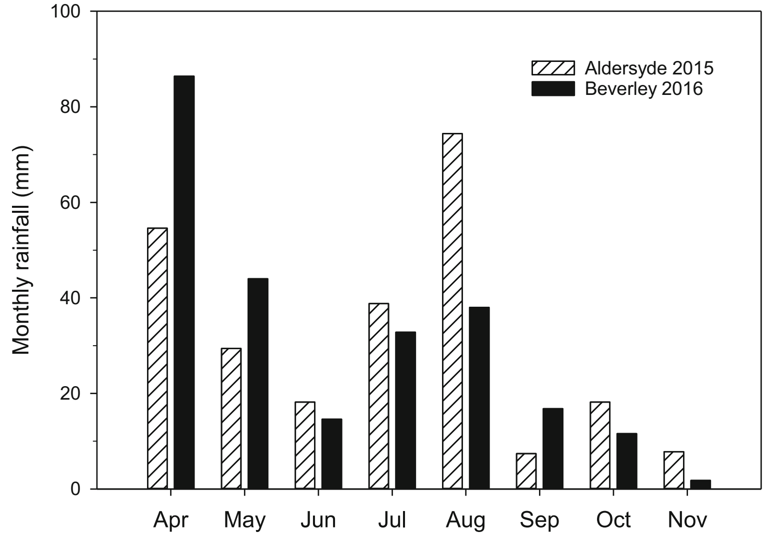

Fig. 1 Monthly rainfall of the growing seasons at Aldersyde in 2015 and at Beverley in 2016. In both years, there were four sowings at every two weeks with the first sowing in mid-April. All plants were harvested in late November

Crop management

In both experiments, weeds were controlled by applying 118 g pyroxasulfone ha^{-1}, 2.5 L trifluralin ha^{-1}, 300 g diuron ha^{-1} and 1.5 L glyphosate ha^{-1} before emergence, plus 1 L Velocity® ha^{-1} and 300 mL Prosaro® ha^{-1} post-emergence. Pests were controlled by applying 1 L chlorpyrifos ha^{-1} and 250 mL bifenthrin ha^{-1} before emergence.

Measurements

Rainfall during the growing season (April to November) was recorded at the experimental sites in both years.

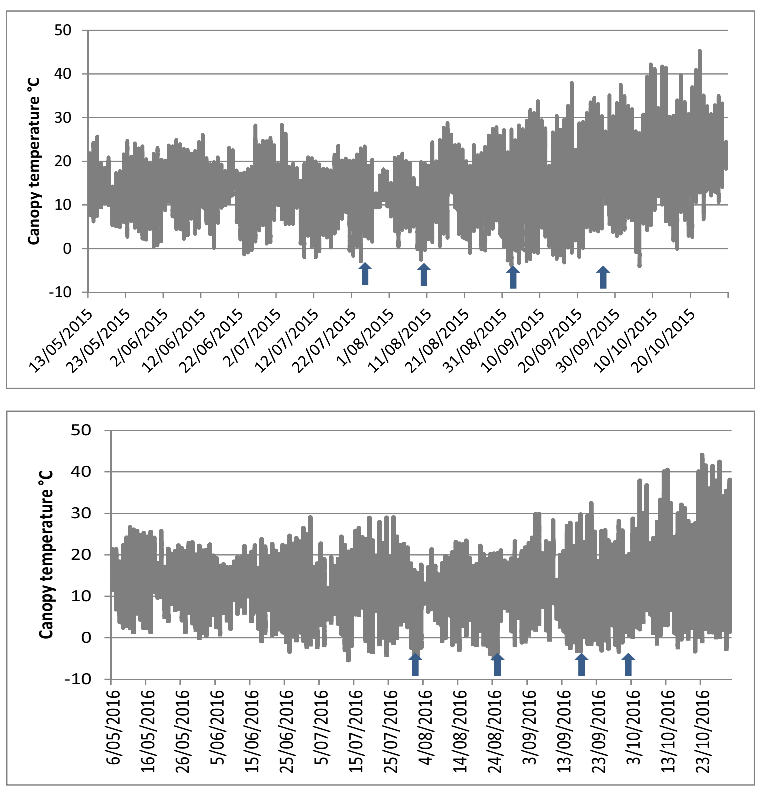

Fig. 2 Canopy temperatures at 600 mm above the ground through the growing season at Aldersyde in 2015 and at Beverley in 2016. The arrows indicate the times when flowering heads tagged for assessing floret sterility of the four sowings in each year

Table 2 Effects of soil potassium (K) and foliar micronutrients (MN) on floret sterility (%, n = 4 and 30 ears plot^{-1}) in wheat cvs. Mace and Wyalkatchem at four times of sowing (TOS 1, 15 April; TOS 2, 29 April; TOS 3, 15 May; TOS 4, 2 June) in 2015

Cultivar	K (kg ha^{-1})	TOS 1	TOS 2	TOS 3	TOS 4
Mace	0	20	39	25	3
	80	19	33	16	3
	80 + MN	21	21	19	4
Wyalkatchem	0	19	35	28	4
	80	15	32	19	4
	80 + MN	14	30	20	3
P-value	Cultivar	0.01	n.s	n.s.	n.s.
	Nutrient	n.s.	0.003	0.05	n.s.
	Interaction	n.s.	n.s.	n.s.	n.s.

n.s. = not significant ($P > 0.05$). Plants at TOS 4 were mostly free from frost damage. Foliar micronutrients were a mixture of 0.5% $CuSO_4$, 1% $MnSO_4$, 1% $ZnSO_4$ and 0.075% Na_2MoO_4

Canopy temperatures for the four-sowing crops were measured at 15 min intervals through the growth stages using Tinytag TGP-4017 temperature loggers (Gemini Data Loggers Ltd., West Sussex, England). The temperature loggers were installed facing north with the internal sensor upwards and secured to a white 50 mm PVC pipe at 600 mm off the ground (anticipated height of wheat heads). Daytime temperatures were not analysed due to inconsistent shading of loggers. In addition, an onsite weather station (Hobo Data Loggers Australia) recorded temperature in a Stevenson screen. Temperatures below 2 °C at the screen or 0 °C at the canopy height were defined as a frost event (Smith et al. 2017). Following frost events, 30 heads per plot reaching anther dehiscence in the middle of the heads were tagged and floret sterility (FS) of the outside florets (discarding the top and bottom florets) was measured five to six weeks later during grain fill (Z85) (Biddulph et al. 2015). The FS values were expressed as a percentage reduction in grain numbers of the total outside-florets of 30 heads in each plot.

At the time of tagging heads for FS assessment, the fully expanded flag leaves were also sampled for K and micronutrient analyses. About 1 g of ground leaf materials was digested in 0.5 M nitric acid in a Milestone microwave (CEM Mars5; CEM Corp., Matthews, NC, USA) after the method of Huang et al. (2004). The concentrations of leaf K and micronutrients were determined by inductively coupled plasma atomic emission spectroscopy (VISTA Simultaneous ICP-AES spectroscopy; Varian, Palo Alto, CA, USA).

The rates of net photosynthesis and stomatal conductance of the fully expanded flag leaves were determined using the LCpro+ advanced photosynthesis system (ADC BioScientific Ltd., Hoddesdon, UK) between 11 am and 2 pm on sunny days from anthesis to grain-fill.

Following a frost event at anthesis, fully expanded flag leaves were collected and immediately frozen in liquid nitrogen and then stored in −80 °C freezer. Frozen leaf samples (~0.5 g) were homogenised in

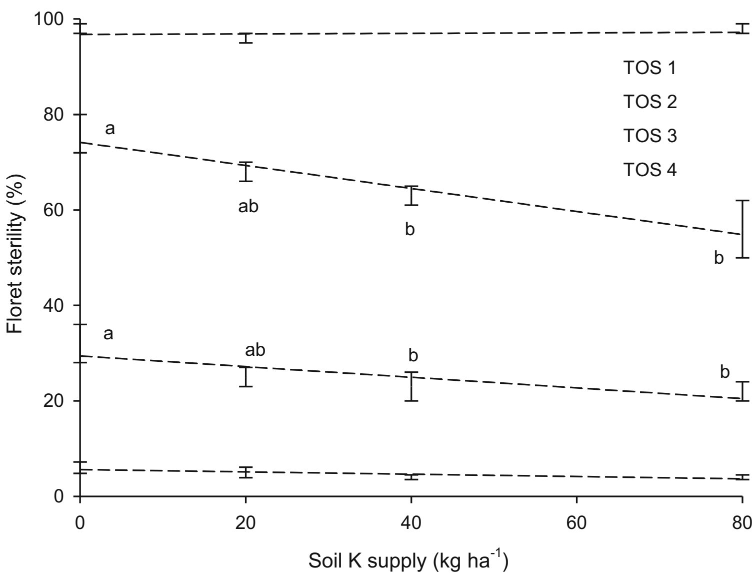

Fig. 3 Floret sterility of wheat cv. Mace at different rates of K supply in the 2016 season. Flowering plants from time of sowing 1 (TOS 1), TOS 2 and TOS 3 experienced severe frost events, while the plants at TOS 4 largely escaped frost events

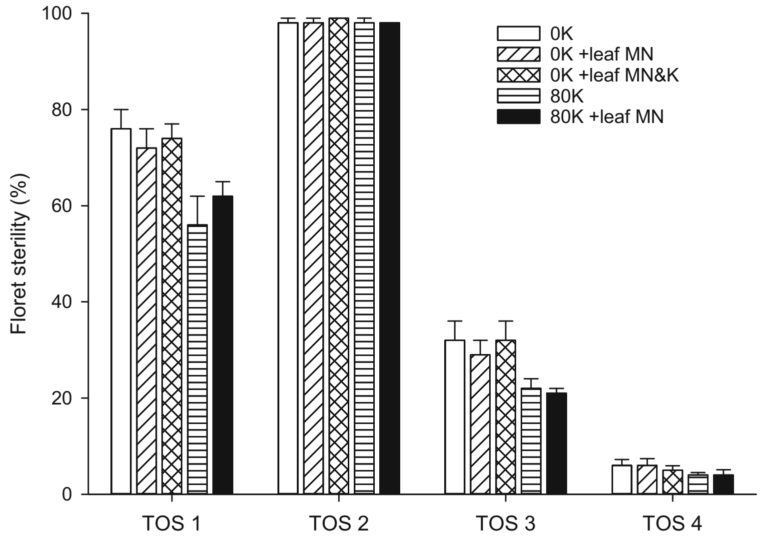

Fig. 4 Compared with soil K (kg ha^{-1}), foliar spray of K (5% K_2SO_4) and micronutrients (MN; 0.5% H_3BO_3, 0.5% $CuSO_4$, 1% $MnSO_4$, 1% $ZnSO_4$, 0.075% Na_2MoO_4) at booting and ear emergence of wheat cv. Mace did not provide extra protection from frost damage in the 2016 season

2 mL of ice-cold buffer (50 mM Na-PO_4, pH 6.8) with a mortar and pestle for 2 min. The homogenates were centrifuged at 14000 *g*, 4 °C for 15 min to obtain the enzyme extracts. The activity of superoxide dismutase (SOD) was measured by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971) with some modifications. An aliquot of 100 μL extract was added to a 3-mL mixture of 50 mM Na-PO_4 (pH 7.8), 0.66 mM EDTA-Na_2, 10 mM methionine, 30 μM NBT and 3.3 μM riboflavin. The reaction occurred under illumination of 350 μmol m^{-2} s^{-1} for 15 min. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT at 560 nm. The SOD activity was expressed as AU g^{-1} fresh weight.

At anthesis and maturity, plant shoots randomly selected from three 0.5-m rows in each plot were excised at the soil surface and dried in a forced-draught oven at 60 °C for 72 h for dry weight. The K concentrations of the whole shoots at anthesis were also measured. In each plot, grain yield at the standard moisture content of 12.5% was machine-harvested. Yield components (ears m^{-2}, grains ear^{-1}, single grain weight) and harvest index (the ratio of grain yield to total aboveground dry matter) were measured by quadrat cuts.

Table 3 Effects of soil potassium (K) and foliar micronutrients (MN) on flag leaf K concentration (%, n = 4) at anthesis of wheat cvs. Mace and Wyalkatchem at four times of sowing (TOS 1, 15 April; TOS 2, 29 April; TOS 3, 15 May; TOS 4, 2 June) in 2015

Cultivar	K (kg ha^{-1})	TOS 1	TOS 2	TOS 3	TOS 4
Mace	0	2.82	2.38	2.01	1.66
	80	3.01	2.61	2.33	1.92
	80 + MN	3.07	2.59	2.30	1.96
Wyalkatchem	0	2.93	2.17	2.03	1.68
	80	3.07	2.46	2.26	1.99
	80 + MN	3.16	2.46	2.30	2.08
P-value	Cultivar	n.s.	0.01	n.s.	n.s.
	Nutrient	0.04	<0.001	<0.001	<0.001
	Interaction	n.s.	n.s.	n.s.	n.s.

n.s. = not significant ($P > 0.05$). Foliar micronutrients were a mixture of 0.5% $CuSO_4$, 1% $MnSO_4$, 1% $ZnSO_4$ and 0.075% Na_2MoO_4

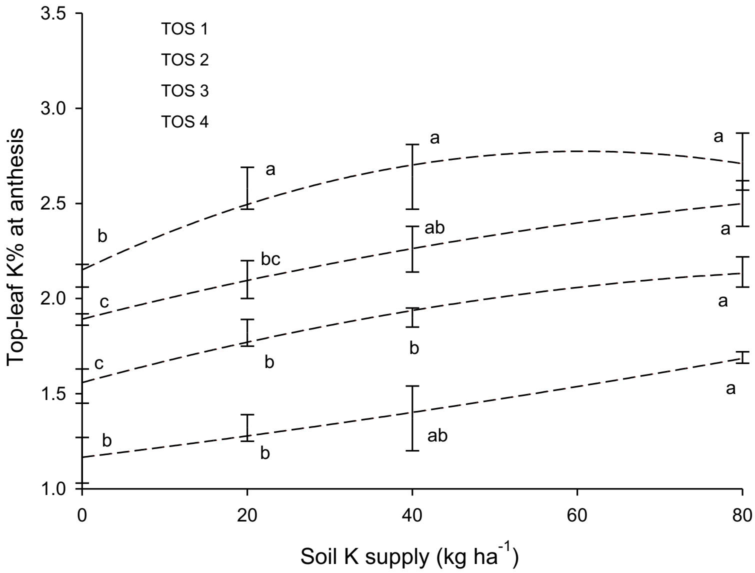

Fig. 5 The concentration of K in flag leaves at anthesis of wheat cv. Mace with different K rates in 2016. Within each time of sowing (TOS), means ($n = 4$) with the same letter are not significantly different at $P = 0.05$. Capped lines are ± standard errors

Statistical analysis

The data from each of the four sowings in the 2015 experiment were subjected to two-way analysis of variance (ANOVA) to assess the effects of wheat genotypes and K treatments and their interactions. The data from each of the four sowings in the 2016 experiment were analysed by one-way ANOVA for plant response to different K rates. Treatment differences were separated by Fisher-protected l.s.d. test and accepted at $P \leq 0.05$. Statistical analyses were conducted by GenStat 10 (Laws Agricultural Trust, Rothamsted, UK).

Results

Frost damage

Total water supply (rainfall + irrigation) during the growing season (April to November) was 274 mm (TOS 1, 2, 3) and 249 mm TOS 4 at Aldersyde in 2015 and 246 mm at Beverley in 2016 (Fig. 1). With June rainfall of just 16–18 mm in both years, the months of September to November were also dry with total rainfall of 33 mm in 2015 and 30 mm in 2016. In the 2015 season, flowering plants at sowings 1–3 experienced frost events especially the

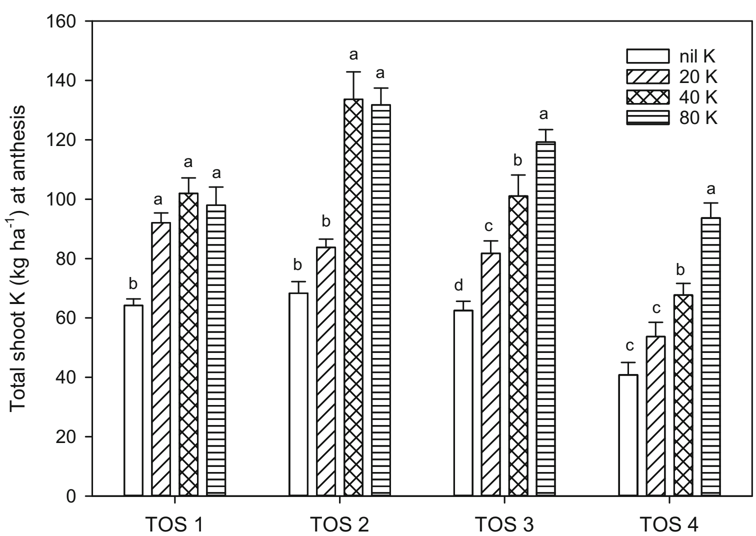

Fig. 6 Total shoot K contents at anthesis of wheat cv. Mace with different K rates (kg ha^{-1}) in 2016. At each time of sowing (TOS), means (n = 4) with the same letter are not significantly different at $P = 0.05$. Capped lines are standard errors

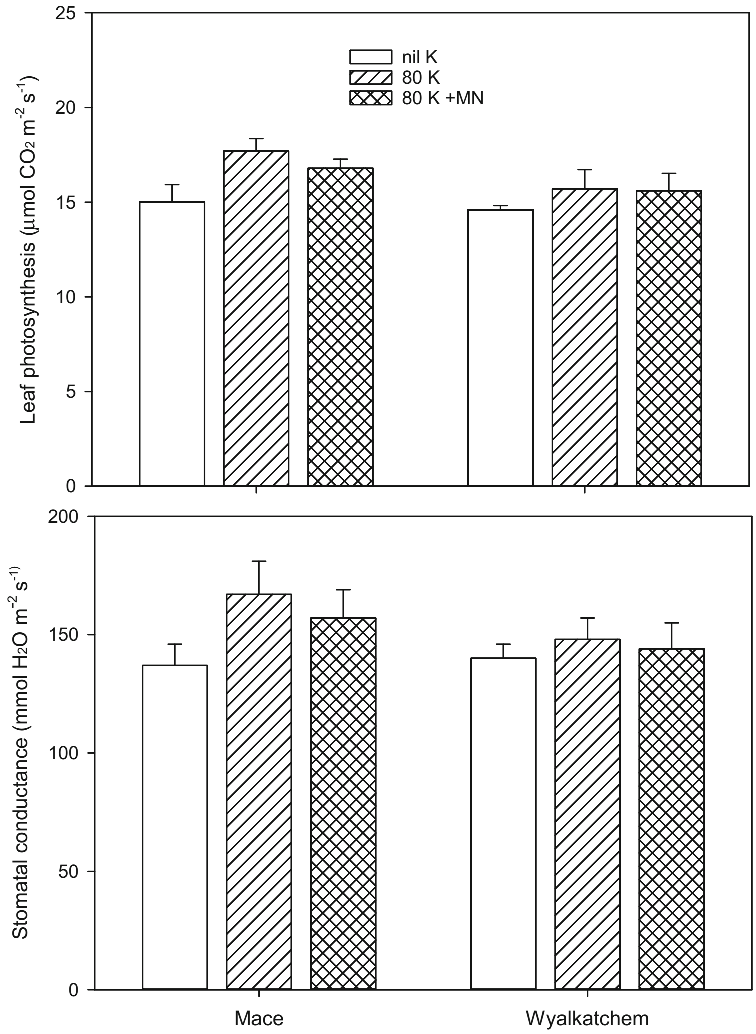

Fig. 7 Photosynthetic gas exchange at anthesis of wheat cv. Mace and Wyalkatchem treated with soil K (kg ha^{-1}) and foliar micronutrients (MN; 0.5% $CuSO_4$, 1% $MnSO_4$, 1% $ZnSO_4$, 0.075% Na_2MoO_4) in 2015. Both leaf photosynthesis and stomatal conductance did not differ between two cultivars, but increased with K supply without additional effect of foliar micronutrients

plants at sowing 3, but the plants at sowing 4 largely escaped frost damage at anthesis (Fig. 2a). The number of days at subzero-degree temperatures was five in July, five in August, 12 in September and two in October. The 2016 season was very cold and was the coldest September recorded in Western Australia, and the number of frost days at the experimental site was 16 in July, 15 in August, 16 in September and seven in October (Fig. 2b).

Floret sterility (FS) in 2015 was 15–20% at sowing 1, 20–40% at sowing 2 and 15–30% at sowing 3, compared with only 3–4% at sowing 4 (Table 2). There were no significant effects of K supply or a K by cultivar interaction at sowing 1 on FS. In contrast, soil K supply reduced FS by 8% (from 32% at nil K to 24% at 80 kg K ha^{-1} when averaged across cultivars) at sowings 2 and 3. In 2016, FS responded to soil K rates at sowings 1 and 3, showing that application of 40, 80 kg K ha^{-1}, but not 20 kg K ha^{-1}, significantly reduced FS (Fig. 3). The FS values at nil, 20, 40 and 80 kg K ha^{-1} were 76, 68, 63, 56% at sowing 1 and 32, 25, 23, 22% at sowing 3, respectively. By comparison, FS at sowing 2 in 2016 was >95% and not affected by K supply, and the sterility at sowing 4 was just 4–6% across the K rates. Foliar spray of trace elements (B, Cu, Zn, Mn, Mo) at booting and ear emergence did not change FS in either year (Fig. 4).

Plant K status

The concentrations of K in the dry matter of flag leaves at anthesis decreased with the delay of sowing and were 2.9, 2.2, 2.0, 1.7% in the plants with nil K applied at sowings 1, 2, 3 and 4 in 2015, respectively (Table 3).

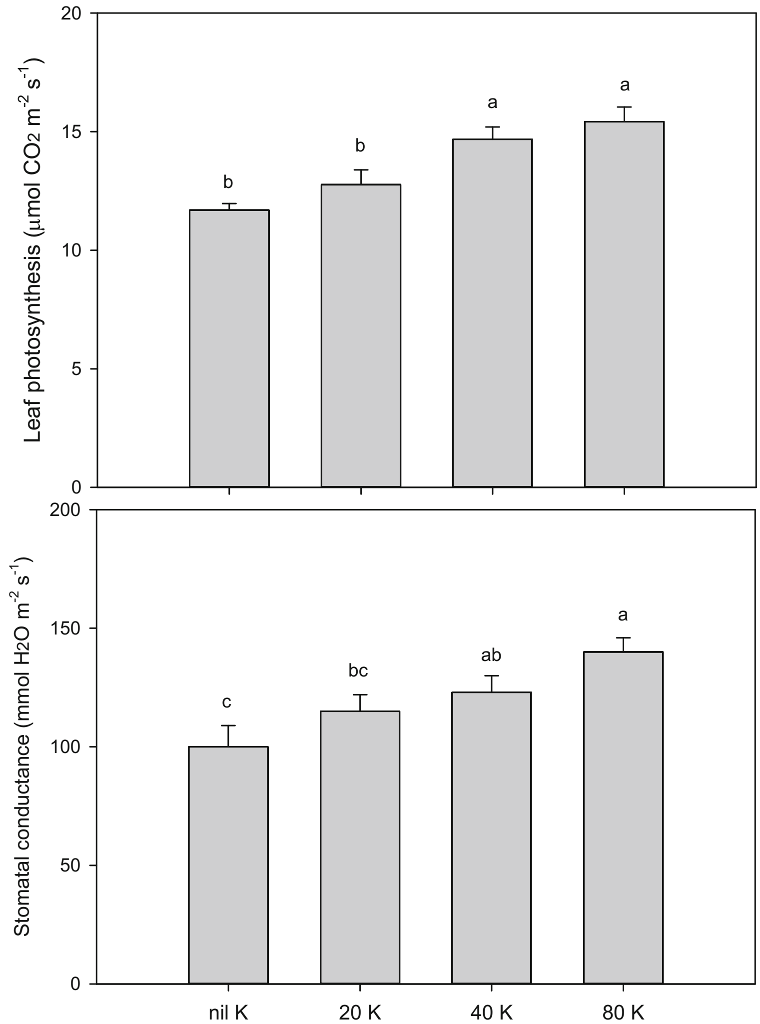

Fig. 8 Photosynthetic gas exchange at anthesis of wheat cv. Mace treated with different rates of soil K (kg ha^{-1}) in 2016

Soil K supply increased leaf K concentrations by 0.3–0.4% across the four sowings, but there were no significant effects of cultivar or cultivar by K interaction.

In 2016, flag-leaf K concentrations at anthesis of the plants with nil K supply were 2.1, 1.9, 1.5, 1.1% at sowings 1, 2, 3 and 4, respectively (Fig. 5). Leaf K concentrations increased with increasing soil K rates, e.g. 2.7, 2.5, 2.1, 1.7% in the plants with 80 kg K ha^{-1} at sowings 1, 2, 3, and 4, respectively. Total shoot K contents at anthesis were also related closely to the rates of K supply (Fig. 6), particularly for the plants in the later sowings.

Photosynthesis and antioxidation

In 2015, soil K supply increased leaf photosynthesis and stomatal conductance at anthesis, but cultivars Mace and Wyalkatchem did not differ in leaf gas exchange. The foliar application of trace elements (Cu, Mn, Zn and Mo) had no additional effect on leaf gas exchange (Fig. 7). In 2016, the plants at nil and 20 kg K ha^{-1} had similar leaf photosynthesis, while 40 and 80 kg K ha^{-1} increased both photosynthesis and stomatal conductance (Fig. 8).

The activity of total superoxide dismutase (SOD), measured for the plants at TOS 1 and 3 on the day following a frost event in 2016, was significantly higher at nil K supply than the plants treated with 20, 40 and 80 kg K ha^{-1} (Fig. 9).

Growth and yield

Compared with nil K supply, 80 kg K ha^{-1} significantly increased shoot dry weights at anthesis and maturity for

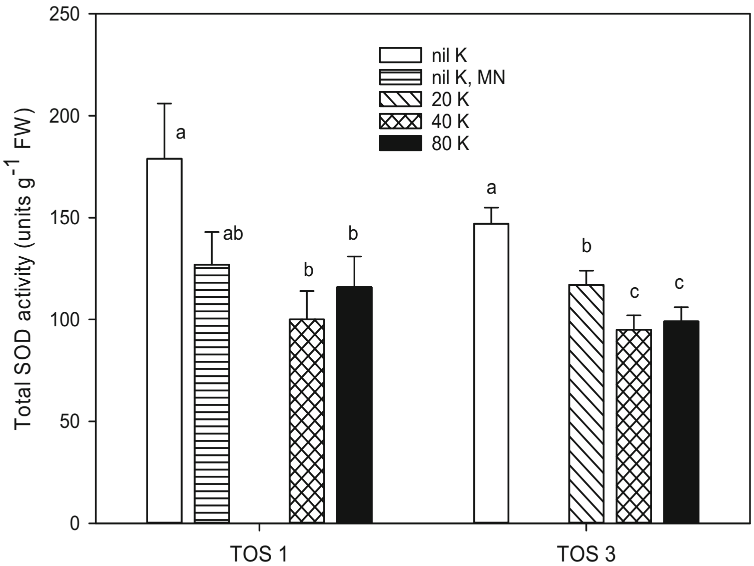

Fig. 9 Effects of soil K (kg ha^{-1}) and foliar micronutrients (MN; 0.5% H_3BO_3, 0.5% $CuSO_4$, 1% $MnSO_4$, 1% $ZnSO_4$, 0.075% Na_2MoO_4) on the activity of total superoxide dismutase (SOD) in flag leaves (time of sowing (TOS) 1 and 3) on the day following a sub-zero frost event in 2016. At each sowing, means ($n = 4$) with the same letter are not significantly different at $P = 0.05$. Capped lines are standard errors

TOS 1 and 2, but not for TOS 3 and 4 in 2015. The cultivars Wyalkatchem and Mace showed similar responses to K supply (Table 4, just the maturity data). In 2016, the Mace plants treated with 40 or 80 kg K ha^{-1} produced greater shoot dry weights than ones with nil, 20 kg K ha^{-1} at TOS 2, 3 and 4 (Fig. 10, just the maturity data).

Soil K supply increased grain yields at TOS 2 and 3 in 2015 (Table 5). The Mace plants had greater grain yields at TOS 1, 3 and 4, due to higher harvest index (data not presented), than the Wyalkatchem plants, but there were no interactions of K by cultivar at any of the four sowings. In 2016, grain yields at TOS 1 and 2 were less than 1 t ha^{-1} and did not differ between the rates of nil, 20, 40 and 80 kg K ha^{-1} (Fig. 11). At TOS 3 and 4, the yields were also similar for the plants with nil, 20 kg K ha^{-1}, but increased in the treatments of 40, 80 kg K ha^{-1}.

Yield components were affected by sowing time in both years. With delay of sowing, ears m^{-2} decreased and grains ear^{-1} increased, but single grain weight showed little change (Table 6). Grain yield and harvest index were significantly higher at TOS 3 and 4 than at TOS 1 and 2. The ear number increased with increasing rate of K supply, but was similar between 40 and 80 kg K ha^{-1}.

Discussion

In southern Australia, frost events are increasing in frequency and severity during the spring coinciding with the sensitive stages of young microspore at pollen development and anthesis in wheat, and is manifest as severe grain yield loss from frost damage (GRDC Grownotes 2016). In this study, K fertiliser application reduced frost damage by reducing FS, which at least partially supports the anecdotal reports from agricultural advisers and growers that wheat crops are more susceptible to frost damage on low K soils. The two sites reported in this study had 41–48 mg Colwell-K kg^{-1} in the 0–10 cm layer, the same as the critical soil K range of 41–49 mg Colwell-K kg^{-1} for wheat

Table 4 Effects of soil potassium (K) and foliar micronutrients (MN) on final biomass (t ha^{-1}, n = 4) of wheat cvs. Mace and Wyalkatchem at four times of sowing (TOS 1, 15 April; TOS 2, 29 April; TOS 3, 15 May; TOS 4, 2 June) in 2015

Cultivar	K (kg ha^{-1})	TOS 1	TOS 2	TOS 3	TOS 4
Mace	0	8.13	7.04	8.18	6.58
	80	9.78	8.71	8.46	6.92
	80 + MN	9.10	8.52	8.30	7.23
Wyalkatchem	0	7.32	7.21	7.48	6.73
	80	9.48	8.58	8.41	7.06
	80 + MN	8.77	8.36	8.18	7.13
P-value	Cultivar	n.s.	n.s.	n.s.	n.s.
	Nutrient	<0.01	<0.01	n.s.	n.s.
	Interaction	n.s.	n.s.	n.s.	n.s.

n.s. = not significant ($P > 0.05$). Foliar micronutrients were a mixture of 0.5% $CuSO_4$, 1% $MnSO_4$, 1% $ZnSO_4$ and 0.075% Na_2MoO_4

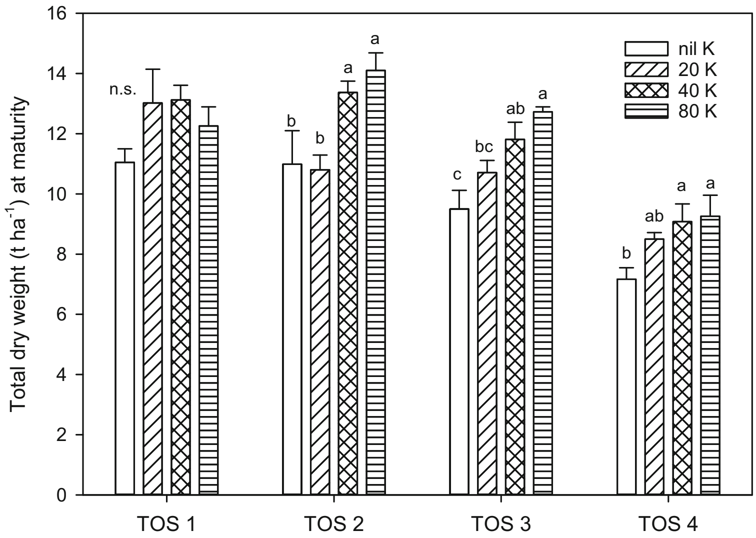

Fig. 10 Total dry weight at maturity of wheat cv. Mace with different K rates (kg ha^{-1}). At each time of sowing (TOS), means ($n = 4$) with the same letter are not significantly different at $P = 0.05$. Capped lines are standard errors

(calculated at 90% of the maximum relative yield; Brennan and Bell 2013). We found that when frost damage was extreme (95% FS) soil K supply had no effect on FS, but with less severe frost events increasing leaf K concentrations in the range of 1.5–2.6% at flowering reduced FS by 10–20% and increased grain yield by 0.2–0.4 t ha^{-1}. The protective role of K in frost damage was also demonstrated by an experiment at Lake Grace in 2016 under severe K deficiency (22 mg Colwell K kg^{-1} soil at 0–30 cm), showing that applying 20 kg K ha^{-1} reduced FS by up to 20% and doubled grain yield in wheat (0.5 t ha^{-1} at nil K to 1.1 t ha^{-1} with K fertiliser) (J. Easton, unpublished). These results suggest that K fertiliser application on deficient or marginal K soils is able to reduce frost damage in wheat and can be part of a comprehensive frost management strategy in the southwest of Western Australia, where 7–50% of the top soils are deficient/ marginal at K levels (Weaver and Wong 2011).

Table 5 Effects of soil potassium (K) and foliar micronutrients (MN) on grain yield (t ha^{-1}, n = 4) of wheat cvs. Mace and Wyalkatchem at four times of sowing (TOS 1, 15 April; TOS 2, 29 April; TOS 3, 15 May; TOS 4, 2 June) in 2015

Cultivar	K (kg ha^{-1})	TOS 1	TOS 2	TOS 3	TOS 4
Mace	0	2.20	1.13	2.69	2.85
	80	2.30	1.28	3.16	3.02
	80 + MN	2.22	1.39	2.91	2.99
Wyalkatchem	0	1.79	1.18	2.51	2.56
	80	1.88	1.27	2.71	2.67
	80 + MN	1.83	1.33	2.65	2.64
P-value	Cultivar	<0.001	n.s.	<0.001	0.001
	Nutrient	n.s.	0.05	<0.01	n.s
	Interaction	n.s.	n.s.	n.s.	n.s.

n.s. = not significant ($P > 0.05$). Foliar micronutrients were a mixture of 0.5% $CuSO_4$, 1% $MnSO_4$, 1% $ZnSO_4$ and 0.075% Na_2MoO_4

The alleviating effect of K on frost damage was previously reported in potato (Grewal and Singh 1980), tomato, pepper and egg plant (Hakerlerler et al. 1997), carnation (Kant and Kafkafi 2002), tropical dry forests at high altitudes (Gómez-Ruiz et al. 2016) and rice (Haque 1988). In the potato experiment on 14 alluvial soils varying in available K from 56 to 158 mg kg^{-1}, frost damage was inversely related to soil available K and leaf K concentrations, indicating the requirement for higher K to increase frost resistance in potato foliage (Grewal and Singh 1980). In rice, spikelet sterility under cold was increased by high nitrogen (N) but decreased by increasing K supply and the K/N ratio in leaves and panicles (Haque 1988). All these findings are largely consistent with the present study on wheat that increasing plant K status, above the critical leaf K concentration of 1.5% at flowering (Reuter and Robinson 1997), increased grain set and yield under field frost conditions. By comparison, in the absence of frost damage, leaf K concentrations <1.5% at flowering predicted yield response to K supply. Hence, high K tissue concentrations can be economically of advantage to the grain growers by acting as an insurance strategy against unexpected environmental events such

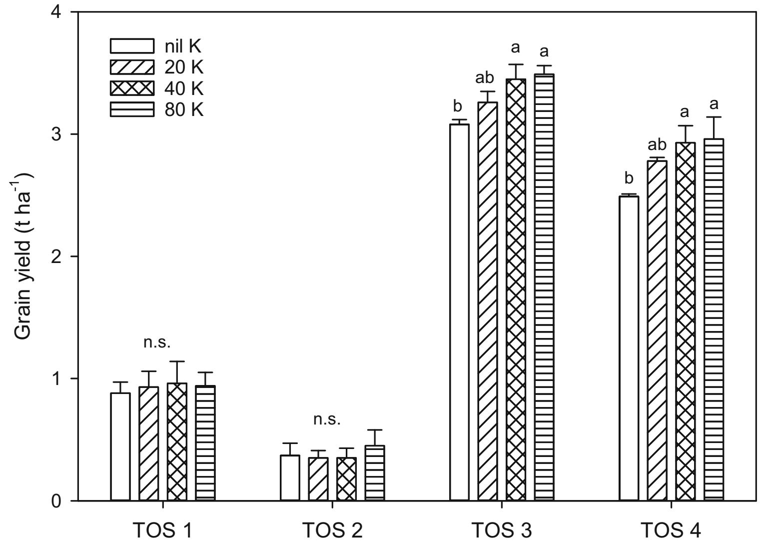

Fig. 11 Grain yield of wheat cv Mace with different K rates (kg ha^{-1}) in 2016. At each time of sowing (TOS), means (n = 4) with the same letter are not significantly different at $P = 0.05$. Capped lines are standard errors

as frost. On the other hand, at low and marginal K tissue concentrations one or more chilling or frost events at the stage of high frost susceptibility, the young microspore stage of pollen development or flowering, may cause severe sterility and yield losses.

Potassium, in addition to its large requirement for plant growth and development, is involved in numerous physiological functions relating to plant tolerance to biotic and abiotic factors such as disease, pest, drought, heat, salt and chilling (Cakmak 2005; Römheld and Kirkby 2010; Oosterhuis et al. 2013). Chilling increases the formation of reactive oxygen species (ROS), which causes photooxidative damage to plant chloroplasts and impairs stomatal conductance and CO_2-fixation capacity (Marschner 1995; Allen and Ort 2001). These cellular targets are also major targets adversely affected by K deficiency (Cakmak 2005). In this study, frost induced an increase in SOD activity by 25–40% in flag leaves of nil K plants compared with the treatments of 20, 40 or 80 kg K ha^{-1} (Fig. 8). Increases in activity of SOD scavenging for superoxide radical ($\cdot O_2^-$) in plants upon exposure to chilling or freezing conditions indicate participation of ROS in chilling-induced cell damage (Foyer et al. 1994; Lee and Lee 2000; Allen and Ort 2001). Therefore, at frost events the K-deficient plants would experience more oxidative stress and frost damage than the plants with K application. While we have only measured SOD in the flag leaf, there may also be an

Table 6 Effects of time of sowing on yield components of wheat crops in 2015 and 2016

Yield component		TOS 1	TOS 2	TOS 3	TOS 4	l.s.d.0.05
Ears m^{-2}	2015	421	291	287	296	25
	2016	351	365	284	287	32
Grains ear^{-1}	2015	11.9	9.4	21.6	21.7	2.2
	2016	8.4	2.2	26.8	26.6	1.2
Single grain wt (mg)	2015	41	46	45	43	3.6
	2016	31	46	44	37	4.7
Grain yield (t ha^{-1})	2015	2.04	1.26	2.77	2.79	0.23
	2016	0.92	0.36	3.35	2.82	0.16
Harvest index	2015	0.24	0.16	0.34	0.40	0.03
	2016	0.08	0.03	0.32	0.34	0.04

In 2015, TOS 1 = 15 April; TOS 2 = 29 April; TOS 3 = 15 May; TOS 4 = 2 June

In 2016, TOS 1 = 13 April; TOS 2 = 4 May; TOS 3 = 20 May; TOS 4 = 10 June increase in oxidative damage in pollen under K deficiency. Moreover, we found that K fertilisation increased leaf photosynthesis by 10% for cv Mace and 5–10% for cv Wyalkatchem and the photosynthetic rates were related to the rates of K applied. Higher photosynthesis with the likely resultant higher tissue sugar contents, together with higher tissue K contents, in the K-applied plants, would enhance the ability of cell osmoregulation and thus frost tolerance by lowering the freezing point of cell sap (Römheld and Kirkby 2010; Wang et al. 2013). In addition, the activities of numerous enzymes which may play a part in frost resistance are dependent on adequate K cytoplasmic concentration (Kant and Kafkafi 2002). The higher ratio of unsaturated to saturated fatty acids in phospholipid rich cell membranes in plants of high K status, can also partially explain increased frost resistance due to enhanced membrane fluidity (McKersie and Leshem 1994; Römheld and Kirkby 2010).

Mineral nutrients other than K also have distinct physiological properties of direct relevance to frost resistance by plants. Boron (B), zinc (Zn) and calcium (Ca) all have roles in stabilizing cell membranes and hence may improve frost resistance (Römheld and Kirkby 2010). Manganese (Mn), copper (Cu) and Zn are constituents in subunits of SOD enzymes, which detoxify oxygen radicals formed under frost and thereby prevent damage to membranes and other cellular constituents (Cakmak 2000; Kant and Kafkafi 2002). In this study, a cocktail of B, Cu, Zn, Mn and Mo was applied by foliar spray at booting and ear emergence but did not affect FS or grain yield in wheat, likely because the experimental sites in both years had adequate topsoil micronutrient concentrations (Table 1). In contrast, our preliminary experiment in canola in 2015 found foliar spray and deep placement of the micronutrients reduced frost-induced pod abortion by 20% compared with the control treatment (unpublished). Similar results are also reported from the studies in East Germany, Ukraine and Russia on winter canola, which showed the mitigating effect of Cu on frost damage when applied as foliar spray, particularly at adequate K supply (reviewed by Römheld and Kirkby 2010). Further work needs to validate the benefits of micronutrient supply in different crops and the interrelationship between micronutrients and K for alleviating frost-induced crop damage to provide improved recommendations of fertiliser management in frost-prone fields.

In summary, this study has shown that K fertiliser application can reduce frost damage to wheat crops and increase grain set and yield unless the frost is too extreme. The extent of alleviation of FS by K supply is associated with leaf K concentrations, photosynthesis and oxidative stress. As effective frost management requires a range of options that enable crops to avoid, tolerate or recover from frost events, K fertiliser use can be one component of the package of frost management solutions in Western Australia where low K soils are widespread and spring frost is becoming more frequent.

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