



# Growth and yield responses of sunflower to drainage in waterlogged saline soil are caused by changes in plant-water relations and ion concentrations in leaves

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## Abstract

**Purpose** While well-designed drainage systems could improve crop growth and yield by mitigating waterlogging and salinity stresses, field evidence of the yield responses to changes in plant-water relations and ion concentrations in leaves is scarce. We investigated the changes in ion concentrations in leaves and plant-water relations of sunflower caused by drainage in waterlogged saline soil, and their relationships to growth and yield.

**Methods** Over two growing seasons, we tested four drainage treatments: undrained, surface drains (SD; 0.1 m deep, 1.8 m apart), subsoil drains (SSD; 0.5 m deep, 4.5 m apart) and SSD+SD. All plots were inundated (2–3 cm depth; water salinity,  $EC_w$ , 1.5–2.5  $dS\ m^{-1}$ ) for 24 h at vegetative emergence and at the 8-leaf stage before opening drains.

**Results** Relative to the most drained treatment (SSD+SD), the undrained treatment caused higher waterlogging at 0–30 cm depth, and decreased solute potential ( $\Psi_s$ ) of soil at 7.5 cm to 52–374 kPa, leaf  $K^+$  by 5–20%, stomatal conductance by 5–37% and leaf greenness by 12–25%, but increased leaf  $Na^+$  by 25–70%,  $Na^+/K^+$  ratio by 38–100% and leaf water potential by 90–250 kPa throughout the cropping season; these changes were closely related to reduced growth and yield.

**Conclusions** The improved yield from the combination of shallow surface and sub-surface drains was attributed to an alleviation of salinity-waterlogging stress early in the season and to increased soil water late in the season that increased  $\Psi_s$  and decreased  $Na^+/K^+$  ratio in leaves.

**Keywords** Cultivar Hysun-33 · Leaf water potential ·  $Na^+/K^+$  ratio in leaf · Relative growth rate ·  $SEW_{30}$  · Solute potential of soil

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## Introduction

Waterlogged saline soils are a serious threat to agricultural productivity (Barrett-Lennard 2003; Falakboland et al. 2017). Globally, around 20% of irrigated areas are affected by secondary salinization, and one-third are facing waterlogging (Dagar and Minhas 2016). Coastal deltas, many of which are important food production regions, are at particular risk because their low elevation, flat terrain and shallow groundwater, which create both waterlogging and salinization of soils (van der Zee Sjored et al. 2017). Waterlogging and salinity affect morphological, physiological, and biochemical processes, seed germination, plant growth, and water and nutrient uptake (Falakboland et al. 2017; Paul et al. 2021c; Wu et al. 2015), resulting in low agricultural productivity, low-income returns and soil degradation (Hu and Schmidhalter 2004).

Waterlogging is a huge challenge to plants, particularly roots. The saturation of soil pores immediately decreases the rates of movement of oxygen into soils (Cannell et al. 1985), decreasing oxygen concentrations in soils (Barrett-Lennard et al. 1986) and consequently decreasing the energy that root tissues can obtain from respiration (Pan et al. 2021).

As separate stresses, waterlogging and salinity have a variety of effects on plant water relations. With waterlogging, the impacts on water relations are variable. Several researchers have reported that waterlogging decreases leaf water potentials ( $\Psi_{\text{leaf}}$ ), i.e. show increased water stress (Ashraf et al. 2011; Shaw 2015). By contrast, there is also evidence that  $\Psi_{\text{leaf}}$  in sunflower and tomato plants can be increased rather than decreased by waterlogging (Bradford and Hsiao 1982; Jackson et al. 1978), but in these cases, was associated with decreased stomatal conductance ( $g_s$ ) (Jackson et al. 1978). One of the immediate effects of salinity is to decrease the availability of water to plant cells and lowering cell water potential ( $\Psi$ ); this decreases cell expansion and growth (Munns 2002). In soil, salinity can be expressed through measures of the solute potential ( $\Psi_s$ ), which increases with soil salinity (in measures like the  $EC_{1:5}$  – the electrical conductivity of a 1:5 soil:water slurry) and decreases in response to soil water content (Paul et al. 2020). In combination, waterlogging and salinity have substantial adverse effects on plant ion relations. A major effect of waterlogging with salinity is the increased

$Na^+$  concentration in shoots, with correspondingly lower  $K^+$  and  $K^+/Na^+$  ratio in the leaf (Barrett-Lennard 2003; Barrett-Lennard and Shabala 2013); these are correlated with reduced photosynthetic rate, stomatal conductance ( $g_s$ ) and shoot growth (Akram et al. 2008; Kirmizi and Bell 2012; Saqib et al. 2005). Part of the differences in responses among studies may be that for many crops, the combination of waterlogging and salinity stresses have more severe impacts on plant growth and yield than either waterlogging or salinity alone (Barrett-Lennard 2003; Barrett-Lennard and Shabala 2013; Falakboland et al. 2017).

The coastal zone of the Ganges Delta covers more than 30% of the total cultivable lands of Bangladesh, and around 40% of this area is affected by various degrees of soil salinity (SRDI 2010). This zone is also subject to waterlogging following heavy rainfall in the monsoon (aman) season, river flooding and the development of a shallow water-table (Mainuddin et al. 2021). In the dry (rabi season) crops can be subject to a wide range of climate risks. Excess soil water after the aman rice harvest can cause waterlogging, which delays the sowing of rabi (dry season) crops, exposing rabi crops to damage because of end of season drought and salinity stresses, thereby increasing the risk of crop failure (Paul et al. 2021a). In addition, heavy rabi season rainfall can also occur, particularly from December to February, which creates waterlogging, another barrier to rabi crop cultivation in this region (Yu et al. 2019). Because of this diversity of risks, most smallholder farms leave their land fallow in the rabi season.

Our study focused on sunflower (Hysun-33), a promising rabi crop in the study area because of its moderate salt-tolerant and drought-adapted features (Elsheikh et al. 2012). Plants use three main strategies to adapt to salt-stress: osmotic stress tolerance, toxic ion ( $Na^+$ ,  $Cl^-$ ) exclusion and tissue tolerance to toxic ions (Munns and Tester 2008). Apart from ions, a range of compatible osmolytes (e.g. proline, glycine betaine, etc.) are involved in osmotic adjustment in plants under salt stress (Yang and Guo 2018). While sunflower is a high-value crop and is becoming a popular rabi crop in this region, it is sensitive to waterlogging like other rabi crops in this area (e.g. mung bean, lentil, sesame, maize and watermelon).

Generally, drainage to alleviate waterlogging and salinity is important for optimal plant growth

and yield. Either surface or subsurface drainage is practiced in many parts of the world to alleviate these constraints. Surface drains can be effective for improving aeration and reducing salinity in the upper root zone of the growing crop (Hou et al. 2016), while deep drains (> 1.75 m) are often recommended for mitigating salinity (Gupta 2002). Islam et al. (2022) found that the combination of shallow surface (10 cm depth) and subsurface drains (50 cm depth) alleviated waterlogging and salinity, and gave a twofold higher sunflower yield than the undrained treatment. While previous field-based studies have reported the effects of drains on waterlogging, salinity, plant morphology and yield (Ritzema et al. 2008; Sharma et al. 2000), they have not reported on related changes in plant relations or ion concentrations in shoots. Hence, while Islam et al. (2022) found that the combination of shallow surface and subsurface drains alleviated waterlogging and salinity and increased sunflower yield, the physiological mechanisms accounting for these responses were not determined. In contrast to previous studies under controlled (net house) conditions in coarse-textured soil, our studies were on the fine-textured soils typical of a large proportion of agricultural land in the Ganges delta. Plants can withstand higher salinity in sandy than in fine-textured soils as sand particles have bigger pores for water to pass through relative to clay particles, leading to rapid salt leaching. Furthermore, clay soils are slower to drain than sandy soils, resulting in more prolonged periods of hypoxia on roots (Warrence et al. 2002).

In the present paper, we investigated the changes in  $\Psi_s$  in soil, plant-water relations and ion concentrations in leaves, and their relation to drainage and sunflower growth and yield that were first reported by Islam et al. (2022). We hypothesized that early in the season, the hypoxia ( $O_2$  deficiency) due to waterlogging and decreased  $\Psi_s$  would lead to increases in the  $Na^+/K^+$  ratio in leaves, decreases in  $\Psi_{leaf}$  and decreases in  $g_s$ , all of which would decrease growth and yield of sunflower. In the first paper (Islam et al. 2022), we also hypothesized that yield damage from the drains would occur due to decreasing soil water content and increasing soil salinity late in the season. This was found not to be true; in this present paper, we present further information to shed light on why this occurred.

## Materials and methods

### Experimental site and season

The methods of the experiments have been previously described in detail by Islam et al. (2022); a brief summary is presented here. The field experiments were undertaken during two consecutive dry (rabi, November–May) seasons in 2018–19 and 2019–20 on a clay-textured soil under waterlogged saline conditions in a farmer's field at Dacope, Khulna, Bangladesh (22.6321° N and 89.5034° E). The experimental site is in the Ganges Tidal Floodplain (Islam et al. 2022), located in the south-western coastal region of Bangladesh. The climate is sub-tropical monsoonal with an average annual rainfall of 1,850 mm, a dry winter (December–February) and a wet summer (March–June) (Rahman et al. 2015). During the first cropping season in 2018–19, total rainfall, monthly average minimum and maximum temperature were 338 mm, 12.4–24.1 °C and 26.9–34.6 °C, respectively, while in 2019–20 they were 54 mm, 13.9–20.6 °C and 24.0–32.3 °C, respectively (Islam et al. 2022). The soil in the experimental field had a clay loam texture with a bulk density of 1.5–1.6 g cm<sup>-3</sup>, a pH of around 8 at 0–60 cm depth and a saturated hydraulic conductivity of 34 mm day<sup>-1</sup> at 0–45 cm depth. The soil EC<sub>1:5</sub> at 0–60 cm depth ranged between 0.2 and 1.0 dS m<sup>-1</sup> during the sunflower growing seasons (Islam et al. 2022).

### Experimental details and crop husbandry

The drain establishment procedure, field layout and crop husbandry have been described in Islam et al. (2022). Sunflower cv. Hysun-33 was used as the test crop. The experiment had 4 drainage treatments, undrained plots, and plots with open surface drains (SD; 0.1 m deep, 1.8 m apart), slotted-pipe subsoil drains (SSD; 0.5 m deep, 4.5 m apart) and SSD + SD treatments. There were three replicates of each treatment. Replicates of the SSD and SSD + SD treatments were blocked together to avoid hydrological interference between treatments: the rationale for this has been previously discussed (Islam et al. 2022).

Each plot was 10 m × 6 m in size and polyethylene sheets were placed vertically around each plot to a depth of 0.6 m to prevent the lateral flow of water from one plot to another plot. A levee of 1 m wide was made between adjacent plots to minimize the cross-flow of water. There were two waterlogging events in a season. The plots

were inundated (2–3 cm above the soil surface) for 24 h at both vegetative emergence (the VE stage of sunflower development, 14 days after sowing) and at the mid-vegetative stage when the crop had 8 leaves (V8 stage, Schneiter and Miller 1981). After inundation for 24 h, drains were opened in the drainage treatments but not in the undrained treatment. The first inundation was supplied artificially using canal water (EC: 1.5–2.5 dS m<sup>-1</sup>), while the second inundation occurred naturally because of heavy rainfall (151 mm on 27–28 February in the first season and 25 mm on 4 January in the second season, Islam et al. 2022).

Sunflower seeds were sown by dibbling on 18 January 2019 and 25 November 2019 with a row to row distance of 60 cm and a plant to plant distance of 30 cm. According to the recommendation of the Bangladesh Agricultural Research Institute (Islam et al. 2022), urea-triple super phosphate-muriate of potash-gypsum-zinc sulphate-boric acid was applied at 200–200–170–170–10–12 kg ha<sup>-1</sup> in both seasons. The crops were harvested at physiological maturity on 29 April 2019 and 19 March 2020 in the first and second seasons, respectively.

### Sampling and measurement techniques

Soil samples for the measurement of EC<sub>1:5</sub>, soil water content (SWC), waterlogging severity and soil solute potential ( $\Psi_s$ ) were collected from the edge and centre of each plot. These were also the locations of plant measurements: stomatal conductance ( $g_s$ ), leaf water potential ( $\Psi_{\text{leaf}}$ ) and achene yield. In the subsoil drainage treatments (SSD and SSD+SD), soil samples were collected from the edge of the drain pipe and midway between two pipes in the centre of the plot. In the SD and control treatments, soils were sampled near the edge and centre of the plot. In addition, three plants in each plot were randomly sampled and composited to one sample for measuring shoot dry weight (SDW) and leaf Na<sup>+</sup> and K<sup>+</sup> concentrations. Dates of sampling are given in the Supplementary Material, Table S5.

### Waterlogging (SEW<sub>30</sub>)

The degree of waterlogging in the soil was quantified by measuring the sum of excess water in the 0–30 cm layer (SEW<sub>30</sub>). Measurements of the depth to water-table were made daily throughout the season, and the

SEW<sub>30</sub> was calculated according to the method of Sieben (1964) and Cox (1988) (see Islam et al. 2022).

### Solute potential

The three soil samples that were collected from each of the positions (centre and edge) at each depth (0–15, 15–30, 30–45, and 45–60 cm) were mixed thoroughly to make a composite sample for each depth. Soil samples were collected at 7 and 14 days after first inundation (DAFI), 10 and 17 days after second inundation (DASI), 30–50% flowering (FL) and harvest (HRV). Soil water content (SWC) was measured gravimetrically (after oven-drying). The EC<sub>1:5</sub> was measured in mixtures of 10 g of air-dried soil with 50 mL of distilled water. The solute potential ( $\Psi_s$ ) of the soil solution was calculated using the following equation (Paul et al. 2020).

$$\Psi_s = -22580 \times EC_{1:5}/W$$

where  $\Psi_s$  is the solute potential (kPa), EC<sub>1:5</sub> is the electrical conductivity (dS m<sup>-1</sup>) of the 1:5 soil:water extract, and W is the soil water content (% w/w).

### Leaf Na<sup>+</sup> and K<sup>+</sup> concentrations and the Na<sup>+</sup>/K<sup>+</sup> ratio

Three plants were randomly selected in each plot; all leaves at 7 DAFI, the 2–3 youngest fully expanded and the oldest live leaf blades at 10 DASI and flowering from each plant were detached from the petiole. Leaves were then rinsed in deionized water, blotted with tissue paper and dried in an oven at 70°C for 72 h. About 0.2 g of ground leaves were digested in a mixture of nitric and perchloric acid (5:2 ratio). Concentrations of Na<sup>+</sup> and K<sup>+</sup> were then measured with a flame photometer (Model: 410, Sherwood) (Yamakawa 1992).

### Stomatal conductance and leaf water potential

Stomatal conductance ( $g_s$ ) and leaf water potential ( $\Psi_{\text{leaf}}$ ) were measured with a leaf porometer (SC-1 Leaf Porometer, Decagon Devices, USA) and a Pressure Chamber Instrument (Model-1000, PMS Instrument Company, USA), respectively. In the first season (2019),  $g_s$  was measured at 3 DASI, 10 DASI and 17 DASI, and  $\Psi_{\text{leaf}}$  at 3 DASI and FL. In the second

season (2019–20),  $\Psi_{\text{leaf}}$  was measured at 3 DASI, 10 DASI, 17 DASI and FL. Three plants were selected randomly from each position (the edge and centre in a plot), and one fully expanded youngest leaf from each plant was measured for  $g_s$  and  $\Psi_{\text{leaf}}$ . Measurements were taken between noon and 2 pm.

#### Shoot dry weight and relative growth rate of shoot

Four shoots were collected randomly from each plot before first inundation, 7 DAFI, 14 DAFI, 10 DASI, 17 DASI and FL, and were dried in an oven at 70 °C for 72 h and weighed. The relative growth rate (RGR) for the single shoot was calculated following Hunt (1982).

$$RGR = \frac{\ln(W_2) - \ln(W_1)}{t_2 - t_1}$$

where  $W_1$  and  $W_2$  are shoot dry weights at times  $t_1$  and  $t_2$ , respectively.

#### Statistical analyses

STAR software (version 2.0.1) was used to do the analysis of variance (ANOVA). Regression analyses for different factors were conducted using Jamovi software (version 1.1.9.0) and the graphs were prepared in Microsoft Office 365. One-way ANOVAs were used to test the significance of the effects of the drains on SDW, RGR,  $\text{Na}^+$  and  $\text{K}^+$  concentration in leaves, and the molar ratio of  $\text{Na}^+$  and  $\text{K}^+$  in leaves. The effects of drains and position within the plot on  $g_s$ ,  $\Psi_{\text{leaf}}$  and leaf chlorophyll content (LCC) were measured using two-way ANOVAs. The significance of the effects of the drain on  $\Psi_s$  of soil was determined using three-way (treatment, position and soil depth) factorial ANOVA models that also considered the effects of soil depth as a repeated measure. The comparison of means was made using the least significant difference (LSD) at  $P=0.05$ . Single-factor regression analysis were done to investigate the relationships between achene yield and other parameters (SDW and  $\Psi_s$  of soil,  $g_s$ ,  $\Psi_{\text{leaf}}$ , LCC,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Na}^+/\text{K}^+$  in leaves). Cross correlations between  $\text{Na}^+$ ,  $\text{K}^+$  or  $\text{Na}^+/\text{K}^+$  in leaves and  $\Psi_s$  or  $\text{EC}_{1.5}$  or  $\text{SEW}_{30}$  were also tested in single factor regression analyses.

## Results

In our previous paper (Islam et al. 2022) drainage treatments caused up to 95% increase in achene yield in sunflower relative to the undrained treatment under waterlogged saline conditions in both seasons. The drainage treatments reduced  $\text{SEW}_{30}$  and soil  $\text{EC}_{1.5}$  at 0–60 cm by 40–60 and 20–40%, respectively, relative to the undrained treatment. Increased sunflower yield was significantly ( $P<0.001$ ) correlated with decreased  $\text{SEW}_{30}$  and soil  $\text{EC}_{1.5}$  in the topsoil (0–15 cm). This paper focuses on the physiological causes of these effects. There was consistency between seasons in response to drainage treatments in waterlogged saline soil for all parameters measured (ion concentrations in leaves, leaf chlorophyll content, leaf water potential, soil solute potential and shoot growth rate), but values in the first (2018–19) and second growing season (2019–20) differed slightly due to variations in rainfall, temperature, soil salinity, and in dates of planting, waterlogging and harvesting.

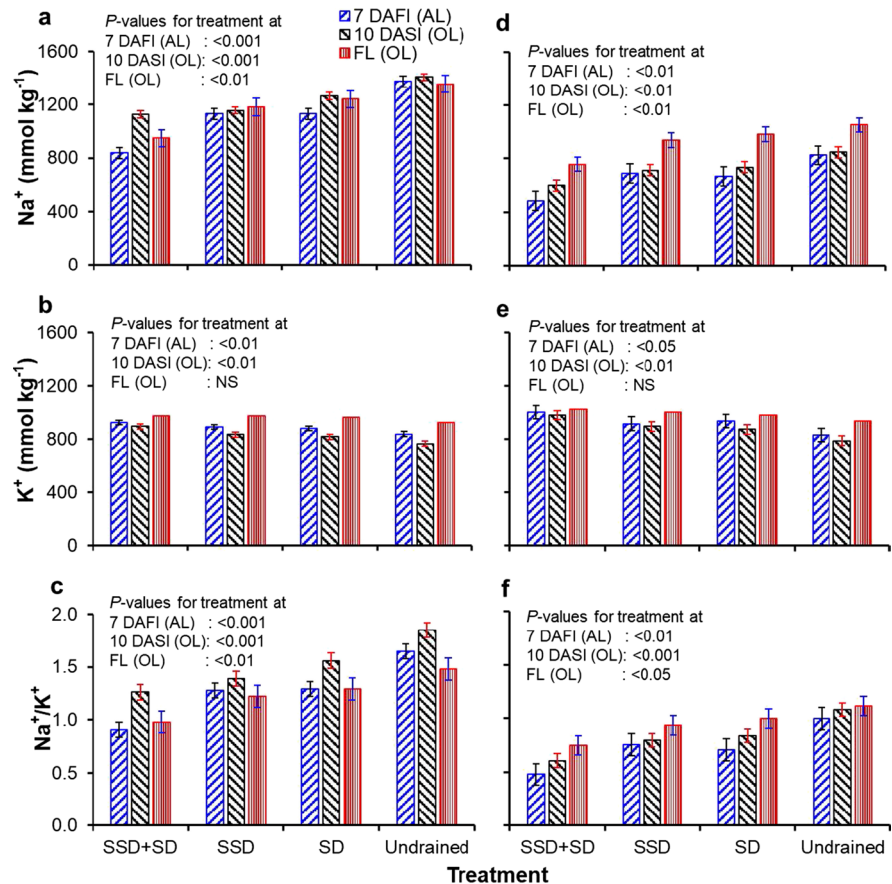
#### Concentrations of $\text{Na}^+$ and $\text{K}^+$ in leaves and their ratio ( $\text{Na}^+/\text{K}^+$ )

This section examines the impacts of treatments on  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Na}^+/\text{K}^+$ , their impacts on yield, and their correlations with other soil factors.

In both seasons,  $\text{Na}^+$  concentration and  $\text{Na}^+/\text{K}^+$  ratio in all leaves at 7 DAFI and in the older leaves at 10 DASI and FL were decreased by drainage treatments compared with the undrained treatment, while the  $\text{K}^+$  concentrations were increased (Fig. 1). At 7 DAFI, the lowest leaf  $\text{Na}^+$  concentration was found in the most drained (SSD+SD) treatment (838  $\text{mmol kg}^{-1}$  in 2018–19 and 485  $\text{mmol kg}^{-1}$  in 2019–20), whereas the highest was with undrained treatment (1374  $\text{mmol kg}^{-1}$  in 2018–19 and 825  $\text{mmol kg}^{-1}$  in 2019–20). The SD and SSD treatments had  $\text{Na}^+$  concentrations between the SSD+SD treatment and the undrained treatment. Similar trends were observed at 10 DASI and FL (Fig. 1). By contrast, leaf  $\text{K}^+$  concentrations at 7 DAFI, 10 DASI and FL were 894–972  $\text{mmol kg}^{-1}$  in 2018–19 and 980–1023  $\text{mmol kg}^{-1}$  in 2019–20 in the most drained treatment and 764–926  $\text{mmol kg}^{-1}$  in 2018–19 and 788–938  $\text{mmol kg}^{-1}$  in 2019–20 with the undrained treatment (Fig. 1). In both seasons, the most drained treatment had the lowest  $\text{Na}^+/\text{K}^+$  ratio at 7 DAFI (all



**Fig. 1** Effect of drains on the concentration of Na and K ions in leaves and its ratio ( $\text{Na}^+/\text{K}^+$ ) in 2018–19 (a–c) and 2019–20 (d–f). Abbreviations: *SSD* subsoil drain, *SD* surface drain, *DAFI* days after first inundation, *DASI* days after second inundation, *FL* flowering, *AL* all leaves, *OL* older leaves. Vertical bars at the top of columns indicate the LSD at 5% level of significance

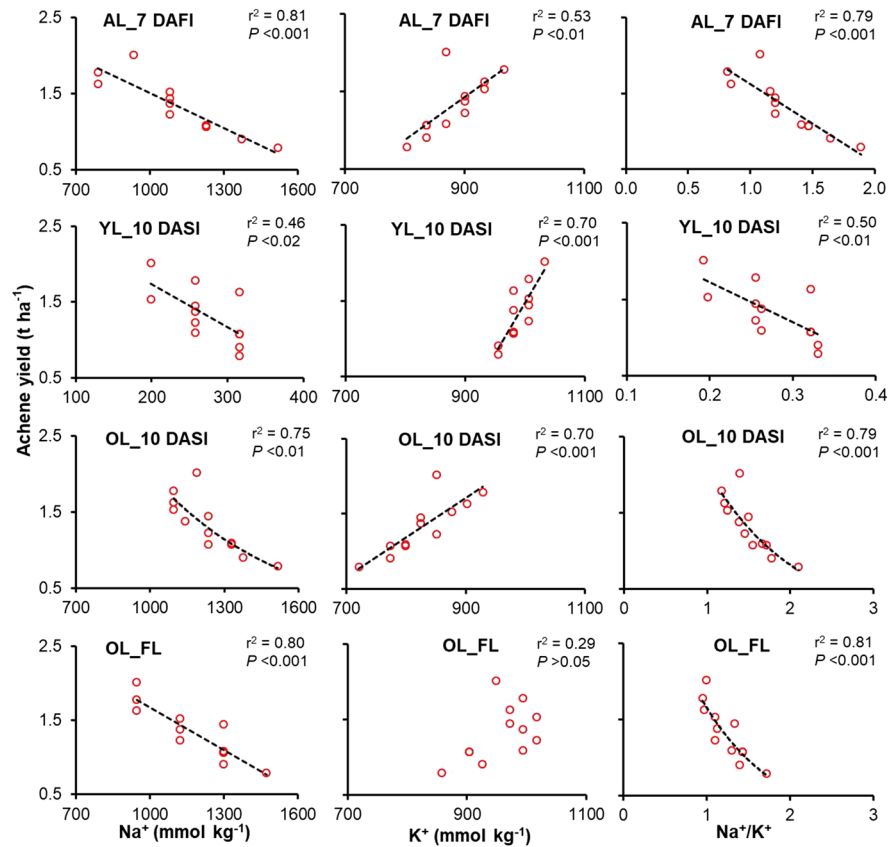


leaves), 10 DASI (older leaves) and FL (older leaves), which was 9–37, 19–32 and 32–52% lower than the SSD, SD and undrained treatments, respectively (Fig. 1). However, younger leaves did not show significant changes in leaf ion concentration or  $\text{Na}^+/\text{K}^+$  ratio (Supplementary Material, Fig. S1).

Leaf  $\text{Na}^+$  concentration and  $\text{Na}^+/\text{K}^+$  ratio during the season were significantly and negatively associated with achene yield, while leaf  $\text{K}^+$  positively correlated with the achene yield (Figs. 2 and 3; yield data reported in Islam et al. 2022). Leaf  $\text{Na}^+$  concentration at different times explained 46–81% and 40–81% of the yield variation in 2018–19 (Fig. 2) and 2019–20 (Fig. 3), respectively. The achene yield variation explained by variation in  $\text{K}^+$  concentration ranged from 53 to 70% in 2018–19 and 50 to 75% in 2019–20. The  $\text{Na}^+/\text{K}^+$  ratio explained 50–79% of the yield variation in 2018–19 and 64–86% in 2019–20. The younger leaves showed weak relationships between ion parameters and achene yield compared with older leaves.

In both seasons, increased  $\text{SEW}_{30}$  and soil  $\text{EC}_{1:5}$  at 0–15 cm, and decreased  $\Psi_s$  at 0–15 cm were significantly correlated with increased  $\text{Na}^+$ , decreased  $\text{K}^+$  and increased  $\text{Na}^+/\text{K}^+$  in leaves at different times during the crop growing season (Table 1). However, younger leaves showed either no relationship or weak relationships, particularly at flowering. The  $\text{SEW}_{30}$  explained 60–76, 52–54 and 56–73% of the variation in 2018–19 and 47–84, 43–79 and 34–89% of the variation in 2019–20 in leaf  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Na}^+/\text{K}^+$  respectively. The strongest correlations with  $\text{Na}^+$  ( $r^2=0.76$ ),  $\text{K}^+$  ( $r^2=0.63$ ) and  $\text{Na}^+/\text{K}^+$  ( $r^2=0.73$ ) were observed in the older leaves at 10 DASI in 2018–19. In 2019–20, leaf  $\text{Na}^+$  ( $r^2=0.84$ ) and  $\text{Na}^+/\text{K}^+$  ( $r^2=0.89$ ) showed similar response but leaf  $\text{K}^+$  ( $r^2=0.79$ ) showed strongest correlation at 7 DAFI. In addition, the longer duration of soil (top 20 cm) saturation was significantly correlated ( $r^2$  values of 0.67–0.90) with the higher  $\text{Na}^+/\text{K}^+$  ratio in leaves in both seasons (Supplementary Material, Fig. S10). In the case of soil  $\text{EC}_{1:5}$ , the  $r^2$  values for  $\text{Na}^+$ ,  $\text{K}^+$  and

**Fig. 2** Correlation between achene yield and  $\text{Na}^+$  or  $\text{K}^+$  or  $\text{Na}^+/\text{K}^+$  in leaves at different times in 2018–19. Abbreviations: *DAFI* days after first inundation, *DASI* days after second inundation, *FL* flowering, *AL* all leaves, *YL* younger leaves, *OL* older leaves. Each point indicates the value from an individual plot ( $n = 12$ )



$\text{Na}^+/\text{K}^+$  were 0.49–0.72, 0.39–0.84 and 0.54–0.74, respectively in 2018–19 and 0.46–0.86, 0.43–0.78 and 0.72–0.90 in 2019–20. The strongest correlations with  $\text{Na}^+$  ( $r^2=0.72$  in 2018–19 and 0.86 in 2019–20) and  $\text{Na}^+/\text{K}^+$  ( $r^2=0.74$  in 2018–19 and 0.90 in 2019–20) were observed in the older leaves at FL in both seasons. The strongest correlation with  $\text{K}^+$  ( $r^2=0.84$ ) were observed in the young leaves at 10 DASI in 2018–19, but in 2019–20 the correlation was strongest ( $r^2=0.78$ ) in the older leaves at 10 DASI.

The soil  $\Psi_s$  also showed significant linear relationship with leaf  $\text{Na}^+$  ( $r^2$  values of 0.58–0.75 in 2018–19 and 0.44–0.85 in 2019–20),  $\text{K}^+$  ( $r^2$  values of 0.46–0.77 and 0.47–0.70 in 2018–19 and 2019–20, respectively) and  $\text{Na}^+/\text{K}^+$  ( $r^2$  values of 0.42–0.78 in 2018–19 and 0.37–0.92 in 2019–20) in both seasons although no correlation was found at 7 DAFI in 2018–19 (Table 1). The strongest correlation with leaf  $\text{Na}^+$  and  $\text{Na}^+/\text{K}^+$  occurred in the older leaves at FL in both seasons. However, the strongest correlation with leaf  $\text{K}^+$  differed between seasons. In 2018–19, it was highest in the young leaves at 10 DASI, while

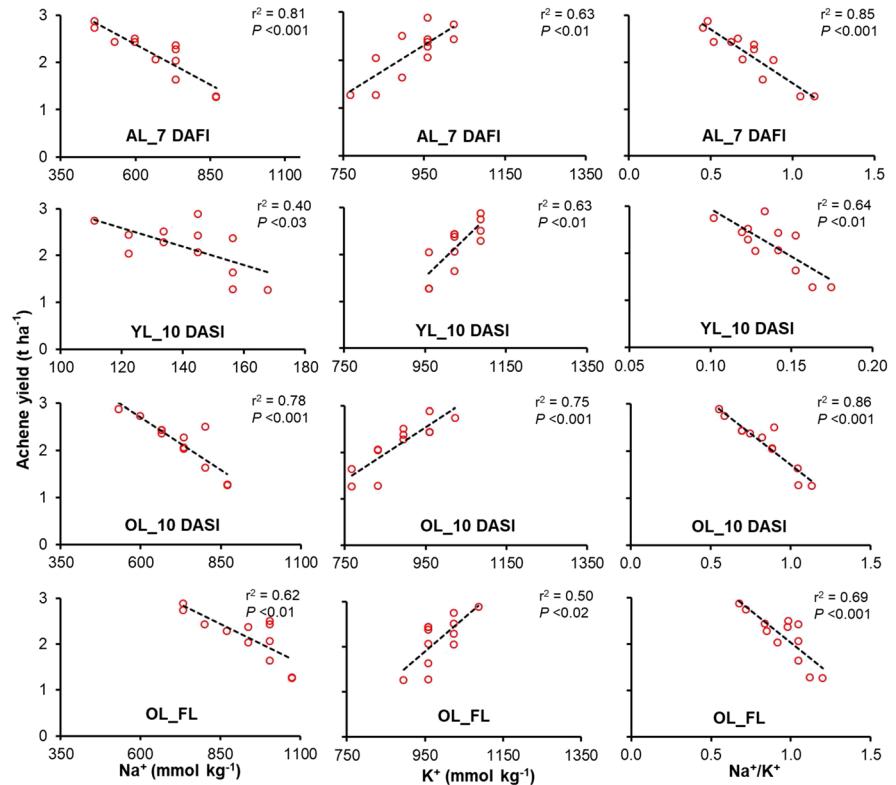
in 2019–20, it was highest in the older leaves at 10 DASI.

#### Stomatal conductance, leaf water potential and leaf chlorophyll content

This section examines the impacts of treatments on stomatal conductance, leaf water potential and leaf chlorophyll content, their impacts on yield, and their correlations with other soil factors.

There was a significant difference ( $P < 0.001$ ) in stomatal conductance ( $g_s$ ) between the treatments at 3 DASI but no difference at 10 and 17 DASI (Table 2). The combined drain treatment (SSD+SD) showed the highest  $g_s$  ( $633 \text{ mmol m}^{-2} \text{ s}^{-1}$ ), while the lowest  $g_s$  was with the undrained treatment ( $401 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) at 3 DASI, compared with the  $g_s$  of  $552 \text{ mmol m}^{-2} \text{ s}^{-1}$  for SSD and  $562 \text{ mmol m}^{-2} \text{ s}^{-1}$  for SD treatments. Drainage treatments also influenced the leaf water potential ( $\Psi_{\text{leaf}}$ ) at different times in both seasons (Table 3). In 2018–19, the lowest  $\Psi_{\text{leaf}}$  was with the SSD+SD treatment ( $-1.29$  to  $-1.40 \text{ MPa}$ ) during the season, while the highest  $\Psi_{\text{leaf}}$  was

**Fig. 3** Correlation between achene yield and  $\text{Na}^+$  or  $\text{K}^+$  or  $\text{Na}^+/\text{K}^+$  in leaves at different times in 2019–20. Abbreviations: *DAFI* days after first inundation, *DASI* days after second inundation, *FL* flowering, *AL* all leaves, *YL* younger leaves, *OL* older leaves. Each point indicates the value from an individual plot ( $n = 12$ )



with the undrained treatment (-1.18 to -1.25 MPa). There was no difference between SD and SSD treatments. A similar trend occurred in 2019–20; the lowest  $\Psi_{\text{leaf}}$  (-1.27 to -1.58 MPa) was with the most drained treatment and the highest  $\Psi_{\text{leaf}}$  (-1.16 to -1.33 MPa) was with the undrained treatment.

Measurements of leaf chlorophyll content (LCC) are reported in the Supplementary Materials (Table S1) with the units of chlorophyll content index (CCI). In 2018–19, the most drained treatment had the highest LCC (12.0–17.2 CCI), the undrained treatment had the lowest LCC (9.0–14.8 CCI), and the SD and SSD treatments had values between the undrained treatment and SSD+SD treatment (Supplementary Material, Table S1). In 2019–20, the relative responses of LCC to drainage treatments were similar to the previous year.

The achene yield was significantly associated with  $g_s$  (positive correlation; one year of data only),  $\Psi_{\text{leaf}}$  (negative correlation) and LCC (positive correlation) in both seasons (Supplementary Material, Table S2). The  $g_s$ ,  $\Psi_{\text{leaf}}$  and LCC accounted for 17–68, 45–67 and 47–69% of the variation in achene yield, respectively. In most cases, the greatest variation was observed at 3 DASI at  $P < 0.001$ .

Soil  $\Psi_s$ ,  $\text{EC}_{1:5}$  at 0–15 cm soil depth and  $\text{SEW}_{30}$  all showed significant relationships with  $g_s$ , LCC and  $\Psi_{\text{leaf}}$  at different times during the cropping season (Supplementary Material, Table S3). The  $\Psi_s$  in soil was positively correlated with  $g_s$  ( $r^2 = 0.45$  at 17 DASI) and LCC ( $r^2$  values of 0.33–0.67), and negatively correlated with  $\Psi_{\text{leaf}}$  ( $r^2$  values of 0.44–0.63). The strongest correlations with  $g_s$ , LCC and  $\Psi_{\text{leaf}}$  were at 17 DASI, FL and FL, respectively. In contrast, the soil  $\text{EC}_{1:5}$  gave a negative correlation with  $g_s$  ( $r^2 = 0.34$  at 17 DASI) and LCC ( $r^2$  values of 0.59–0.74), and a positive correlation with  $\Psi_{\text{leaf}}$  ( $r^2$  values of 0.42–0.63). The strongest relationships with  $g_s$ , LCC and  $\Psi_{\text{leaf}}$  were at 17 DASI, FL and FL, respectively. The  $\text{SEW}_{30}$  also showed negative correlation with  $g_s$  ( $r^2$  values of 0.19–0.80) and LCC ( $r^2$  values of 0.57–0.90), and a positive correlation with  $\Psi_{\text{leaf}}$  ( $r^2$  values of 0.25–0.68). The highest  $r^2$  values were observed at 3 DASI. The results also showed that plant height (Supplementary Material, Fig. S2) and leaf area (Supplementary Material, Fig. S3) were negatively correlated with  $\Psi_{\text{leaf}}$  at 3 DASI and at FL in both seasons. Plant height and leaf area explained 58–64% and 56–72% of the variation in  $\Psi_{\text{leaf}}$ , respectively.



**Table 1** Significance of effects of SEW<sub>30</sub>, soil EC<sub>1:5</sub> at 0–15 cm and Ψ<sub>s</sub> at 0–15 cm on Na<sup>+</sup>, K<sup>+</sup> and Na<sup>+</sup>/K<sup>+</sup> in leaves at different times during the growing season in 2018–19 and 2019–20

	Significance level with r <sup>2</sup> values and direction of the slope (in brackets)					
	2018–19			2019–20		
	SEW <sub>30</sub> (cm days)	EC <sub>1:5</sub> (dS m <sup>-1</sup> )	Ψ <sub>s</sub> (kPa)	SEW <sub>30</sub> (cm days)	EC <sub>1:5</sub> (dS m <sup>-1</sup> )	Ψ <sub>s</sub> (kPa)
<b>At 7 DAFI</b>						
Na <sup>+</sup> (AL)	(+) 0.74***	(+) 0.57**	NS	(+) 0.81***	(+) 0.72***	(-) 0.63**
K <sup>+</sup> (AL)	(-) 0.52**	(-) 0.41*	NS	(-) 0.79**	(-) 0.59**	(+) 0.47*
Na <sup>+</sup> /K <sup>+</sup> (AL)	(+) 0.73***	(+) 0.60**	NS	(+) 0.88***	(+) 0.73***	(-) 0.61**
<b>At 10 DASI</b>						
Na <sup>+</sup> (YL)	NS	(+) 0.49*	(-) 0.58***	(+) 0.47*	(+) 0.46*	(-) 0.44*
K <sup>+</sup> (YL)	(-) 0.54**	(-) 0.84***	(+) 0.77***	(-) 0.43*	(-) 0.72***	(+) 0.57**
Na <sup>+</sup> /K <sup>+</sup> (YL)	NS	(+) 0.54**	(-) 0.62**	(+) 0.61**	(+) 0.73***	(-) 0.65**
Na <sup>+</sup> (OL)	(+) 0.76***	(+) 0.62**	NS	(+) 0.84***	(+) 0.60**	(-) 0.48*
K <sup>+</sup> (OL)	(-) 0.63***	(-) 0.74***	(+) 0.53**	(-) 0.71***	(-) 0.78***	(+) 0.70***
Na <sup>+</sup> /K <sup>+</sup> (OL)	(+) 0.73***	(+) 0.69***	(-) 0.42*	(+) 0.89***	(+) 0.72***	(-) 0.60**
<b>At FL</b>						
Na <sup>+</sup> (YL)	NS	NS	NS	NS	NS	NS
K <sup>+</sup> (YL)	NS	(-) 0.49*	(+) 0.46*	NS	(-) 0.43*	(+) 0.48*
Na <sup>+</sup> /K <sup>+</sup> (YL)	NS	NS	NS	(+) 0.34*	NS	(-) 0.37*
Na <sup>+</sup> (OL)	(+) 0.60**	(+) 0.72***	(-) 0.75***	(+) 0.70***	(+) 0.86***	(-) 0.85***
K <sup>+</sup> (OL)	NS	(-) 0.39*	(+) 0.42*	NS	(-) 0.57**	(+) 0.60**
Na <sup>+</sup> /K <sup>+</sup> (OL)	(+) 0.56**	(+) 0.74***	(-) 0.78***	(+) 0.68***	(+) 0.90***	(-) 0.92***

DAFI days after first inundation, DASI days after second inundation, FL flowering, AL all leaves, YL younger leaves, OL older leaves, Ψ<sub>s</sub> solute potential in soil, \* = P < 0.05, \*\* = P < 0.01, \*\*\* = P < 0.001, NS = non-significant. For all relationships n = 12

**Table 2** Effects of drains on stomatal conductance at different times in 2018–19

Treatment	Stomatal conductance (mmol m <sup>-2</sup> s <sup>-1</sup> )		
	3 DASI	10 DASI	17 DASI
SSD+SD	633 a	944	955
SSD	552 b	946	952
SD	562 b	937	947
Undrained	401 c	900	898
P-value	<0.001	NS	NS

Means with the same letter are not significantly different at 5% level of significance. Abbreviations: SSD subsoil drain, SD surface drain, DASI days after second inundation

**Solute potential**

This section examines the impacts of treatments on solute potential (Ψ<sub>s</sub>) in the soil, and their impacts on yield.

The drainage treatments and soil depths significantly influenced Ψ<sub>s</sub> in the soil in 2018–19 (Fig. 4) and 2019–20 (Fig. 5). In both seasons, there were significant interactions between drainage treatments and soil depth, but no interaction between treatment and position in the plot, or between depth and position in the plot. In general, the range in Ψ<sub>s</sub> values between treatments was greatest in shallow soil, with these differences increasing with time. The Ψ<sub>s</sub> values were lowest with the undrained treatment (-148 to -614 kPa in 2018–19; -75 to -555 kPa in 2019–20), highest with the SSD+SD treatment (-79 to -482 kPa in 2018–19; -51 to -300 kPa in 2019–20), with the SD and SSD treatments were between these extremes.

The Ψ<sub>s</sub> was lowest in the upper soil (average depth 7.5 cm), increasing sharply at average depth 22.5 cm. At average depth 7.5 cm, the higher Ψ<sub>s</sub> was with SSD+SD treatment (-87 to -482 kPa) and the lower values were with the undrained treatment (-177 to -614 kPa) throughout the season in both seasons. The SSD and SD treatments had a similar Ψ<sub>s</sub> in the topsoil (average depth

**Table 3** Effects of drains on leaf water potential at different times in 2018–19 and 2019–20

Treatment	Leaf water potential (MPa)							
	2018–19				2019–20			
	3 DASI	10 DASI	17 DASI	FL	3 DASI	10 DASI	17 DASI	FL
SSD+SD	-1.29 c	-	-	-1.27 b	-1.29 b	-1.35 b	-1.58 c	-1.27 b
SSD	-1.22 ab	-	-	-1.19 ab	-1.22 a	-1.27 a	-1.44 b	-1.19 ab
SD	-1.24 b	-	-	-1.22 b	-1.25 ab	-1.29 ab	-1.47 b	-1.22 b
Undrained	-1.18 a	-	-	-1.16 a	-1.20 a	-1.25 a	-1.33 a	-1.16 a
<i>P</i> -value	<0.01	-	-	<0.001	<0.01	<0.05	<0.001	<0.001

Means with the same letter are not significantly different at 5% level of significance. Abbreviations: *SSD* subsoil drain, *SD* surface drain, *DASI* days after second inundation, *FL* flowering

7.5 cm), but at average depth 22.5 cm, the SSD treatment had higher  $\Psi_s$  than the SD treatment. In the deeper soil (average depth 52.5 cm), there was a little variation in  $\Psi_s$  between the treatments.

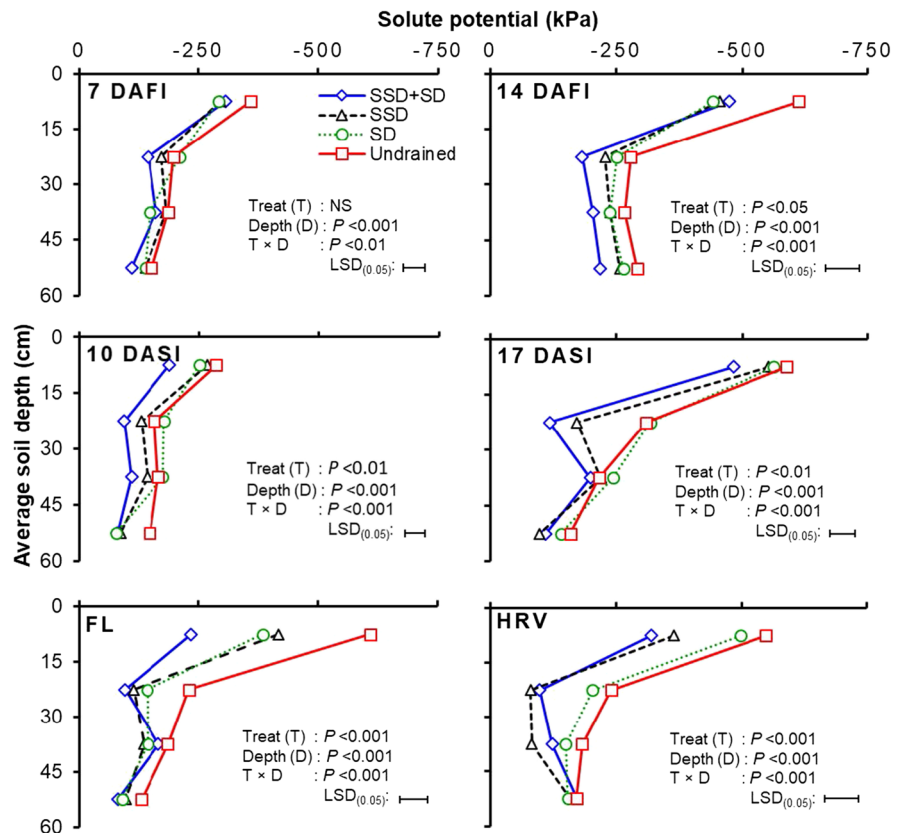
Figure 6 shows the relationship between sunflower yield and  $\Psi_s$  measured at 0–15 cm depth on 6 occasions during each growing season. The 12 relationships shown were all significant, but accounted for most variation at

flowering (with  $r^2$  values of 0.92 in 2018–19 and 0.77 in 2019–20 (Fig. 6).

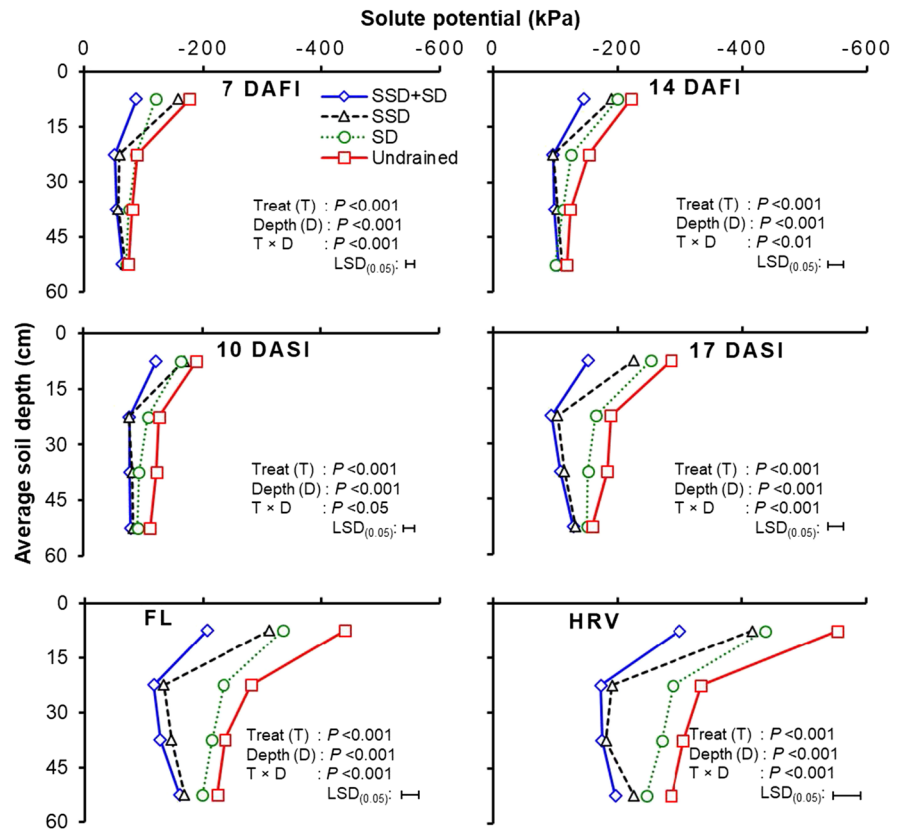
Relative growth rate and dry weight of shoot

The dry weight data used to calculate the relative growth rate (RGR) of the shoots are reported

**Fig. 4** Effect of different drains on solute potential at different soil depths at different times during the crop growing season in Dacope, Bangladesh, in 2018–19. Abbreviations: *SSD* subsoil drain, *SD* surface drain, *DAFI* days after first inundation, *DASI* days after second inundation, *FL* flowering, *HRV* harvest. Each point is the average of 3 replicates



**Fig. 5** Effect of different drains on solute potential at different soil depths at different times during the crop growing season in Dacope, Bangladesh, in 2019–20. Abbreviations: SSD subsoil drain, SD surface drain, DAFI days after first inundation, DASI days after second inundation, FL flowering, HRV harvest. Each point is the average of 3 replicates



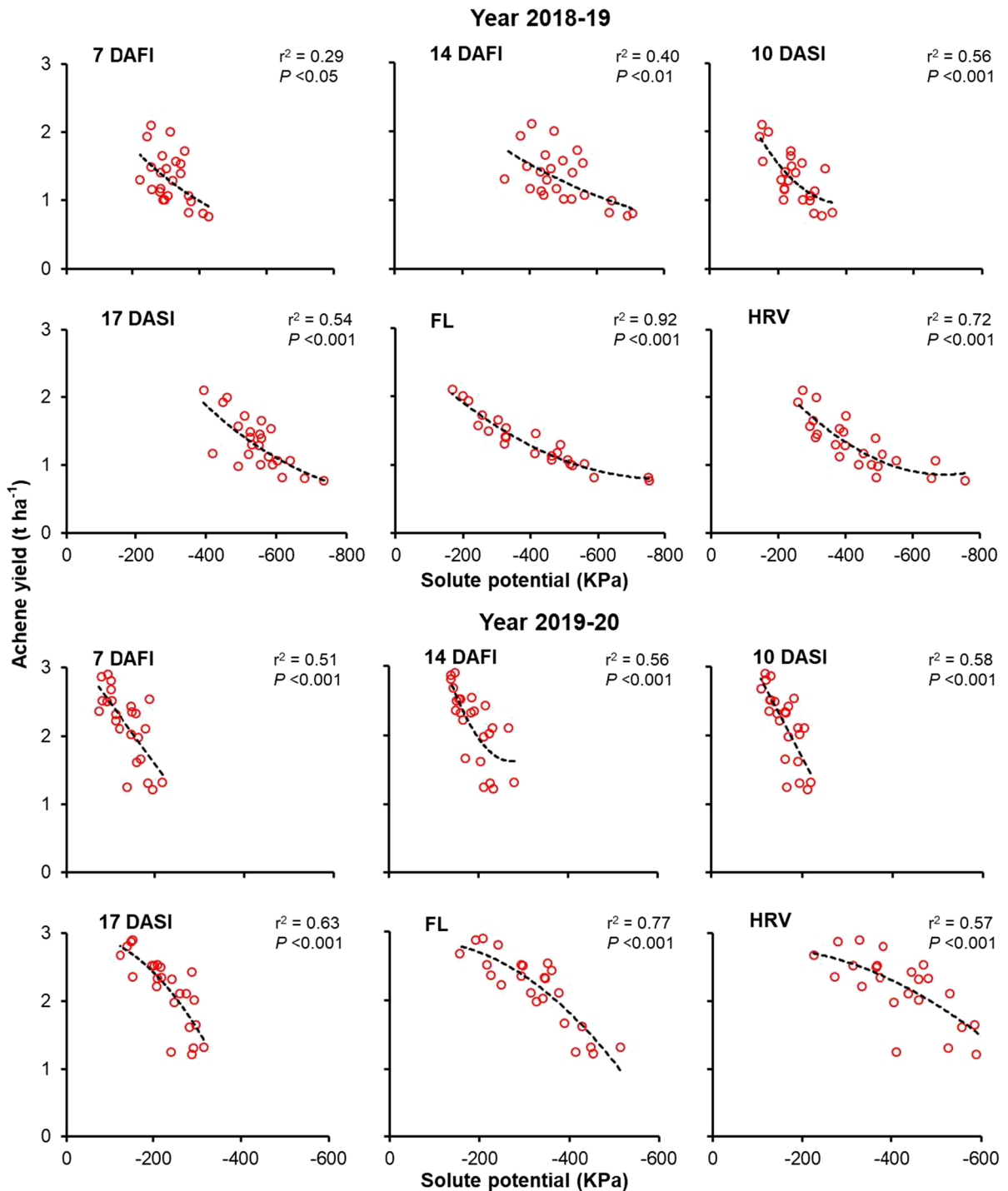
in Supplementary Materials (Table S4). RGR was measured over 4 time intervals: before inundation to 7 DAFI, 7 DAFI to 14 DAFI, 10 DASI to 17 DASI and 17 DASI to flowering (Fig. 7). In each growing season, RGR measurements showed a curvilinear response with time, with values being greatest between 7 to 14 DAFI; this was also the interval that maximised the differences between treatments, with highest RGR in the most drained treatment (0.17 and 0.18  $\text{g g}^{-1} \text{d}^{-1}$ ) and lowest RGR in the undrained treatment (0.11 and 0.11  $\text{g g}^{-1} \text{d}^{-1}$ ). From 17 DASI to flowering, there was no variation between treatments (Fig. 7).

In both seasons, the shoot dry weight showed strong negative correlations with  $\text{SEW}_{30}$  ( $r^2$  values of 0.85–0.95 in 2018–19, and 0.82–0.94 in 2019–20) and soil  $\text{EC}_{1.5}$  ( $r^2$  values of 0.66–0.93 in 2018–19, and 0.69–0.84 in 2019–20) (Supplementary Material, Figs. S4 and S5). In contrast, there were positive linear relationships with  $\Psi_s$  in soil, with  $r^2$  values of 0.41–0.90 and 0.52–0.86 in 2018–19 and 2019–20, respectively (Supplementary Material, Fig. S6). In

addition, the higher shoot dry weights were significantly correlated with higher leaf  $\text{K}^+$  ( $r^2$  values of 0.46–0.73 in 2018–19, and 0.47–0.80 in 2019–20), lower leaf  $\text{Na}^+$  ( $r^2$  values of 0.78–0.80 in 2018–19, and 0.47–0.82 in 2019–20) and higher  $\text{Na}^+/\text{K}^+$  ratio ( $r^2$  values of 0.34–0.78 in 2018–19, and 0.65–0.90 in 2019–20) (Supplementary Material, Figs. S7 and S8). The LCC also showed positive linear relationships with shoot dry weight, with  $r^2$  values of 0.75–0.89 and 0.62–0.80 in 2018–19 and 2019–20, respectively (Supplementary Material, Fig. S9).

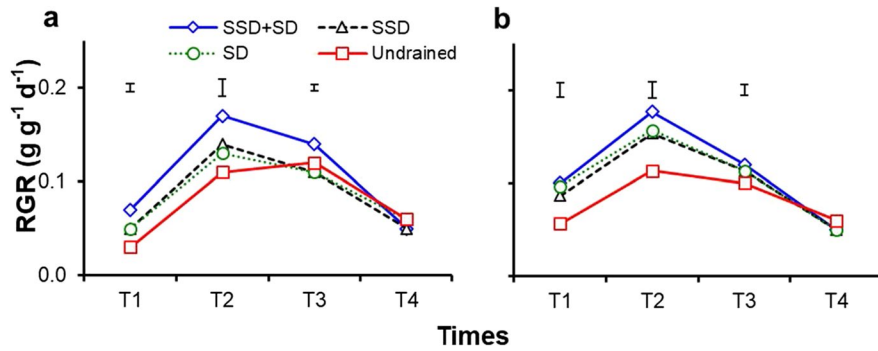
## Discussion

In our previous paper focusing on the yield of sunflower in waterlogged saline soils (Islam et al. 2022), we reported that a combination of shallow drains (SSD with 0.5 m depth and SD with 0.1 m depth) yielded 20–37, 16–45 and 92–95% higher achene weight than SSD, SD and undrained treatments, respectively. Here we investigated the



**Fig. 6** Relationship between sunflower yield and solute potential at 0–15 cm soil depth at different times during the crop growing period in Dacope, Bangladesh, in 2018–19 and 2019–20. Abbreviations: *DAFI* days after first inundation, *DASI* days

after second inundation, *FL* flowering, *HRV* harvest. Each point indicates values from an individual plot taken at the centre or edge of the plot ( $n=24$ )



**Fig. 7** Effect of drains on the RGR of the shoot during the cropping season in: (a) 2018–19, and (b) 2019–20. Abbreviations: RGR=relative growth rate, T1=before inundation to 7 days after first inundation (DAFI), T2=7 DAFI to 14 DAFI,

T3=10 days after second inundation (DASI) to 17 DASI and T4=17 DASI to flowering. Each point is the mean of 3 replicates. Vertical bars at each time indicate the LSD at 5% level of significance

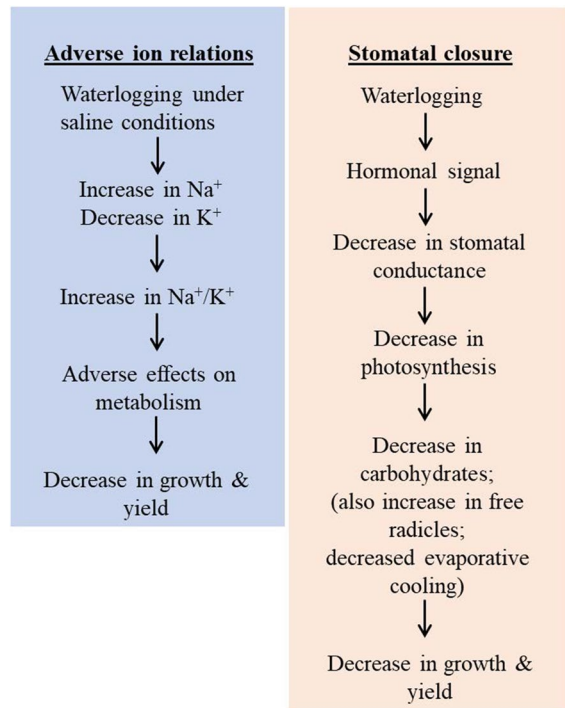
possible physiological mechanisms behind these yield responses in field-grown sunflower. We found that the positive responses in growth and yield were correlated with less hypoxia in the soil, higher (less negative)  $\Psi_s$ , and in the plant – improved ion relations (lower leaf  $\text{Na}^+$ , higher leaf  $\text{K}^+$  and lower  $\text{Na}^+/\text{K}^+$  ratio) but higher  $g_s$ , and lower  $\Psi_{\text{leaf}}$ . This discussion has two sections that focus on the causes of growth responses to drainage early in the growing season immediately after the two inundation events, and then to different mechanisms during the recovery period towards the end of the growing season.

Effects of hypoxia and reduced soil solute potential early in the growing season

This section focuses on two sequences of physiological changes (causes and effects) that occurred early in the growing season. We have termed these the ‘adverse ion relations’ and ‘stomatal closure’ sequences (Fig. 8).

*Adverse ion relations*

In saline soils that waterlog, drainage has the potential to overcome adverse ion relations in plants (i.e., increased  $\text{Na}^+$ , decreased  $\text{K}^+$ , increased  $\text{Na}^+/\text{K}^+$ ) in two ways: by improving soil aeration thereby overcoming adverse waterlogging-salinity interactions (c.f. Barrett-Lennard 2003; Barrett-Lennard and



**Fig. 8** Schema of possible mechanisms of growth and yield reduction under waterlogged saline conditions early in the season

Shabala 2013) and by decreasing soil salinity (i.e., increasing  $\Psi_s$ ) (c.f. Munns 2002). In overview, our data suggest that the beneficial effects of drainage on ion relations occur initially through the first of these mechanisms, and later through the second.



The initial cause of the beneficial effect of drainage derives from the analysis of Barrett-Lennard (2003) and Barrett-Lennard and Shabala (2013). The most important consequence of waterlogging in the field is to reduce the oxygen ( $O_2$ ) concentration due to the replacement of air with water, low rates of  $O_2$  solubility in water, and rapid utilization of dissolved  $O_2$  by roots and microorganisms (Barrett-Lennard et al. 1986; Belford et al. 1985; Cannell et al. 1985). Although we did not measure the concentration of oxygen or the redox potential of the soil, it is evident from earlier studies (Barrett-Lennard et al. 1986) that the rhizosphere (10–20 cm) loses ~75% of its dissolved  $O_2$  within 2–5 days after the commencement of waterlogging. This hypoxic condition causes widespread metabolic disruption including reductions in  $H^+$ -ATPase pumping activity, disturbance of cytosolic  $K^+$  homeostasis, disturbance to the transport of essential nutrients, and ultimately tissue death, starting first with the root tips (Barrett-Lennard and Shabala 2013; Colmer and Greenway 2011; Wu et al. 2021; Barrett-Lennard et al. 1988). There is now a substantial body of published evidence that suggests that when root-zones become waterlogged (i.e., hypoxic), plants rapidly reduce the selectivity of  $K^+$ / $Na^+$  uptake in favour of  $Na^+$  and obstruct  $K^+$  transport to the shoots (Armstrong and Drew 2002), which increases the concentration of  $Na^+$  and decreases  $K^+$  in the leaf tissues, leading to reduced plant growth and yield (Barrett-Lennard and Shabala 2013).

In our study, strongest evidence for the strong initial effects of hypoxia on changes to  $Na^+$  and  $K^+$  uptake comes from comparisons of the most drained treatment (SSD+SD) with the undrained treatment. By 7 days after the first inundation, plants with the SSD+SD and undrained treatments would have experienced a water-table less than 20 cm deep for 1–2 and 4–5 days, respectively. (Capillarity into soil pores shallower than this depth would have ensured that these soils would have been hypoxic.) This 3-day difference in hypoxia between the undrained and SSD+SD treatments was associated with 64 and 70% increases in the concentration of  $Na^+$  (Fig. 1a, d) and 9 and 16% decreases in the concentration of  $K^+$  in leaves (Fig. 1b, e); the speed of these changes (within 7 days) suggests that the effects were caused mostly by the interaction between hypoxia and salt in the growth medium: typically, the effects of hypoxia on  $Na^+$  and  $K^+$  concentrations in leaves can be evident

after just a few days (see reviews by Barrett-Lennard 2003; Barrett-Lennard and Shabala 2013) whereas changes in ion concentrations in leaves because of variation in soil salinity takes longer (weeks, months) (see review by Munns 2002).

In the longer term, there would also have been effects on ion concentrations in leaves caused through beneficial effects of drainage on  $\Psi_s$  of the soil (c.f. Paul et al. 2020). The  $\Psi_s$  of the soil is proportional to the salt concentration in the soil and is inversely related to the soil water content of the soil (Rengasamy 2006). In our previous paper (Islam et al. 2022), the shallow combined drain treatment decreased soil  $EC_{1.5}$  at 0–60 cm depth by 35–44%, early in the season (7 DAFI) relative to the undrained treatment. By 7 DAFI, the  $\Psi_s$  was more negative in the undrained treatment (by 41–44 kPa) relative to the most drained treatment and these differences extended throughout the growing season. We argue that these long-term differences in  $\Psi_s$  could have affected ion concentrations in leaves over the longer term (later in the growing season) but would not likely have been responsible for the differences in ion concentrations in leaves immediately after the first inundation. For both of these scenarios, the increased uptake of  $Na^+$  relative to  $K^+$  at the cellular level can disorder enzyme activities affecting a broad range of catabolic and synthetic processes (Tester and Davenport 2003), which might be plausible reasons for reduced crop growth and yield.

We are aware of one other study with sunflower which has reported the effects of hypoxia and salinity on the concentration of  $Na^+$  in plants grown in nutrient solution (Kriedemann and Sands 1984). In this work, the combination of salinity (50 mM NaCl) and hypoxia (bubbling with  $N_2$  gas) from days 24 to 50 increased  $Na^+$  concentration in leaves 35-fold relative to aerated non-saline conditions; by contrast, salinity alone increased  $Na^+$  concentration by 4.5-fold.

One final aspect on ion relations in our study needs comment. In undrained plants at 10 DAS,  $Na^+$  and  $Na^+/K^+$  ratios were around 350–400 and 450–500% higher in older than in younger leaves, respectively. This is supported by an earlier study (de Azevedo Neto et al. 2020) which reported that  $Na^+$  and  $Na^+/K^+$  ratios were about 70 and 585% higher, respectively, in older leaves of sunflower (cv. AG967) relative to younger leaves during the vegetative stage (at 35 days

after germination) under saline conditions (100 mM NaCl for 20 days). The explanation for this is that salts are continuously deposited in leaves through the transpiration stream, and salt accumulation in leaves, therefore, gradually increases with time. Relative to import in the transpiration stream, there is little retranslocation of salt from older leaves. The presence of salt in leaves, already absorbed, therefore continues despite the salt around the root being removed (Munns 2002).

### Stomatal closure

A second impressive physiological change in sunflower following the onset of hypoxia was a decrease in stomatal conductance ( $g_s$ ) in leaves. The  $g_s$  of leaves controls  $\text{CO}_2$  uptake (which affects photosynthesis) and transpiration (which affects water and nutrient uptake) (Farooq et al. 2009). Stomatal closure can also lead to the accumulation in tissues of free radicals/reactive oxygen species (ROS), decreased evaporative cooling, and a decrease in LCC, leading to cell death (Zhang et al. 2017; Zheng et al. 2009).

In the present study, we found decreases in  $g_s$  associated with the undrained treatment early in the season. This lower  $g_s$  in undrained plants was associated with decreased  $\Psi_s$  and higher (0.11–0.25 MPa)  $\Psi_{\text{leaf}}$  compared with the most drained treatment (Table 3). The causes of decreased  $g_s$  were therefore not ion excess in the external medium and consequent adverse water relations in the leaves. Another mechanism must therefore be invoked. Waterlogging is known to decrease the stomatal conductance in a range of dicots including: sunflower (Kriedemann and Sands 1984; Yan et al. 2018), tomato (Bradford and Hsiao 1982; Jackson et al. 2003) and a range of tree species (Pereira and Kozłowski 1977; Schnull and Thomas 2000). Evidence from some of these studies (Jackson et al. 1978; Bradford and Hsiao 1982; Pereira and Kozłowski 1977) shows that these decreases in  $g_s$  were not associated with decreases in  $\Psi_{\text{leaf}}$ , suggesting that the plants communicate the presence of waterlogging to the leaves by means of a chemical/hormonal signal, believed to be abscisic acid (Pan et al. 2021) as suggested in Fig. 8. For example, *HaHB11* might act as a biotechnological tool to improve waterlogging tolerance and crops' yield as it can regulate various genes involved in

glycolysis, sucrose breakdown and fermentation pathways, which are induced by hypoxic conditions (Cabello et al. 2016). It can also regulate other genes (alanine aminotransferase, heat shock proteins, aspartate aminotransferase) induced due to hypoxia. Our observation of a recovery in  $g_s$  within 10 days after waterlogging of sunflower in the field, is consistent with the results from a previous study with *Fraxinus pennsylvanica* that stomata closed due to waterlogging began to reopen after 15 days; in that study, the recovery in  $g_s$  was associated with the formation of new adventitious roots (Sena Gomes and Kozłowski 1980).

Effects of solute potential late in the season, leading to decreases in plant growth and yield

Generally, at the end of the dry rabi season (March–April), soil salinity increases and SWC decreases in the study area (Rahman et al. 2015; Salehin et al. 2018). In our first paper (Islam et al. 2022), we hypothesized (incorrectly) that the presence of deep drains (i.e., excess drainage) might dry the soil early in the growing season, decreasing the availability of water late in the growing season, thereby having an adverse effect on crop growth. In fact, the reverse occurred: the most drained treatment had increased SWC which maintained higher (less negative)  $\Psi_s$  values in the soil. How could the drains actually increase SWC? Our observations suggest that the drains appear to be 'watering' the plants. The soils of the Ganges Delta are generally 'shrink-swell' clays (Moslehuddin et al. 1999) that form deep cracks as the soil dries out (c.f. Paul et al. 2021b). It is likely that when rain falls on the soil surface late in the growing season, a large proportion of this water rapidly runs down these cracks. Interception of the cracks by the slotted pipe subsoil drains provides a route for this water to then be rapidly redistributed laterally through a whole plot, where it can then recharge the soil profile to depths greater than 50 cm. It can also re-supply the shallower soil with water by capillarity. At these depths in the bulk of the soil, there is less evaporation, so the water is conserved for later crop growth. By contrast, in undrained soils, there may still be some movement of rainwater to the bottom of cracks, but the water remains in the immediate locality of that crack where it is susceptible to more rapid evaporation, which is exacerbated by the less soil shading due to lower LAI

(Islam et al. 2022; Villalobos and Fereres 1990). We conclude that drained soils experienced better rather than worse water relations at the end of the growing season.

It should be noted however that our explanation for these effects requires the combination of cracks plus drains to harvest late season rainfall. In situations where there was no late season rainfall, the combination of cracks plus drains might be worse for crop yields than in the undrained soils as originally hypothesised. The key question is whether plants late in the season used predominantly rainwater associated with subsurface drains or groundwater or both. The sources of water being used by plants could be investigated by measuring the stable isotope composition of water ( $\delta D$  and  $\delta^{18}O$ ) if the isotopic signatures vary between the groundwater and rainwater. For example, based on different isotopic signatures in groundwater and rainwater, Mensforth et al. (1994) and Thorburn and Walker (1993) concluded that groundwater was the dominant source of water for trees, despite its salinity, but the proportion of groundwater used by trees declined after rainfall.

## Conclusion

In a salt-affected, waterlogged coastal zone clay soil in the Ganges Delta, shallow drains improved sunflower growth and yield by decreasing waterlogging ( $SEW_{30}$ ) and duration of hypoxia, and increasing the  $\Psi_s$  of soil, leaf  $K^+$  and  $g_s$ , and decreasing leaf  $Na^+$  and  $Na^+/K^+$  ratio. However, the  $\Psi_{leaf}$  increased in the undrained treatment. Indeed, decreased  $\Psi_{leaf}$  was significantly correlated with increased LA and plant height. Early in the season, the most drained treatment (SSD + SD) reduced the waterlogging and soil salinity impacts on the plant-water relations and ion concentrations in leaves of sunflower more efficiently than other treatments. In the late-season, shallow combined drains increased  $\Psi_s$  of soil by increasing water availability and reducing soil salinity, resulting in better plant-water and ion relations.

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## Declarations

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