CROP SCIENCE SOCIETY
OF SA INCORPORATED
C/- Waite Campus
PMB No 1, Glen Osmond, South Australia 5064
ABN 68 746 893 290

NEWSLETTER

May 2020

The next Crop Science Society Technical Forum is scheduled for Wednesday the 17th June at Roseworthy from 7.30pm. Details to come in the next newsletter.

Yitpi Foundation Awards – Call for Applications

We are pleased to announce that applications for Yitpi Foundation Awards and Grants-in-Aid are now being requested with a closing date of Monday 15th of June 2020. We would appreciate it if you would please publicise this on your relevant websites and with your colleagues.

Guidelines for applications are attached and grants will fall within the three categories listed below;

1. Crop science research
2. Agricultural education
3. Studies of the linguistics and culture of Australian Aboriginal peoples

Further details are available from jane.rathjen01@gmail.com or mobile 0404 062 734.

Kind regards,

Jane Rathjen
Yitpi Foundation

The 22nd Australasian Weeds Conference has been postponed until October 2021

Member in Focus – Jade Rose

As the newest (I think) member of Crop Science Society I would like to introduce myself to those who do not know me yet and say a huge thanks for allowing me to be on the committee (I'm honoured). I am an early career researcher, who is passionate about agriculture and providing farm relevant research to growers.

I do not come from a farming background but grew up in Macclesfield which is closest to the farming region in Strathalbyn. I have always had a passion for the outdoors and sustainability. Initially I undertook a Bachelor of Environmental Policy and Management along with a Bachelor of Science (double majoring in soil science and ecology). However, I found the science degree and its potential to be applied more interesting than the other degree. After this, I landed a job at SARDI for 2+ years in the pulse and oilseed pathology lab mainly working on ascochyta blight in pulses, I undertook my honours whilst working there on 'comparison of aggressive and non-aggressive *ascochyta lentis* isolates in lentil cultivar PBA Hurricane XT'.

From SARDI, I worked at Hart Field Site-Group last year (2019) as a researcher and regional intern. I am particularly interested in disease and soil. However, extremely interested in learning pretty much anything I can from growers, agronomists and researchers (there is SO much to learn). I personally think it is key to have an understanding of farming systems and industry when working in any agricultural area of research. I ticked off a lot of these things working at Hart such as trial planning, seeding, in season measurements and harvesting and generally interacting and going to growers' farms.

This year, I decided to take on a PhD, my aim of this was to (try) provide some useful information to growers with my project. The current project is investigating "Controls and constraints: nutrient release and nitrogen benefit from above and belowground pulse crop residues in mixed cropping grain systems in southern Australia". Basically, trying to optimize our nitrogen inputs and quantify season to season effects of the N that pulse crops leave in the soil.

Into the future, I hope to continue developing sustainable agricultural practices and research, with applicable impacts. I hope I can give back to CSSSA in some way or form and hope to learn a lot from everyone involved.

Variety mixtures as a strategy for frost risk mitigation by Andy Barr

I was recently asked by a local farmer (near Pinery on the Adelaide Plains, 350 -375mm annual rainfall) to comment on the option of growing a wheat variety mixture to reduce the risk of frost. He was considering mixing Scepter and either Illabo or Nighthawk. I too am planning to grow variety mixtures to reduce my risk of a wipeout from a single frost event. There have been too many significant frosts in our area over the past 5 years to ignore and keep saying that it is an aberration and continue to do the same things.

A long time ago and in a galaxy far away (well 1988-1994 anyway), I did a PhD under Tony Rathjen which spent much of its time looking at variety mixtures and their yield performance as well as their value as a tactic for the control of foliar disease. Frost then was just something that the mallee farmers worried about and so was not a consideration at all in the design of the thesis experiments.

So to respond to the query, I decided to put down some thoughts on variety mixtures gleaned from my thesis and the literature I read (it was a hot topic at the time internationally) and place them in the context of a current need for strategies to reduce the risks of catastrophic losses from frosts at anthesis and in early grain fill.

- My view is that you should only include varieties for which there is a case to grow that variety as a normal (pure) variety on your farm – don't think that I would grow Illabo or Nighthawk here as a variety on its own, whereas Catapult, Rockstar or Cutlass yield well here and are 8-10 days later in flowering compared to Mace. Our growing season here I think is too short for varieties like Illabo and Nighthawk.
- I would look at the range in flowering times in the graph below and select one variety from the short (0-5 days), one from medium (6-10 days) and one from the latter group (11 plus days), so that instead of having a pure variety flowering over a week with the majority of flowers over just 3 days, this mixture will extend flowering time over nearly 3 weeks, reducing your chance of a single frost event having a catastrophic effect on yield. Note that Nighthawk would be 7-10 days later than Trojan when sown in first week of May (from the Longreach data I have seen) and that I estimate would be 3-5 days later than Cutlass (the latest on this chart). Illabo could be even later.

Wheat flowering times (from SARDI Sowing guide 2020)

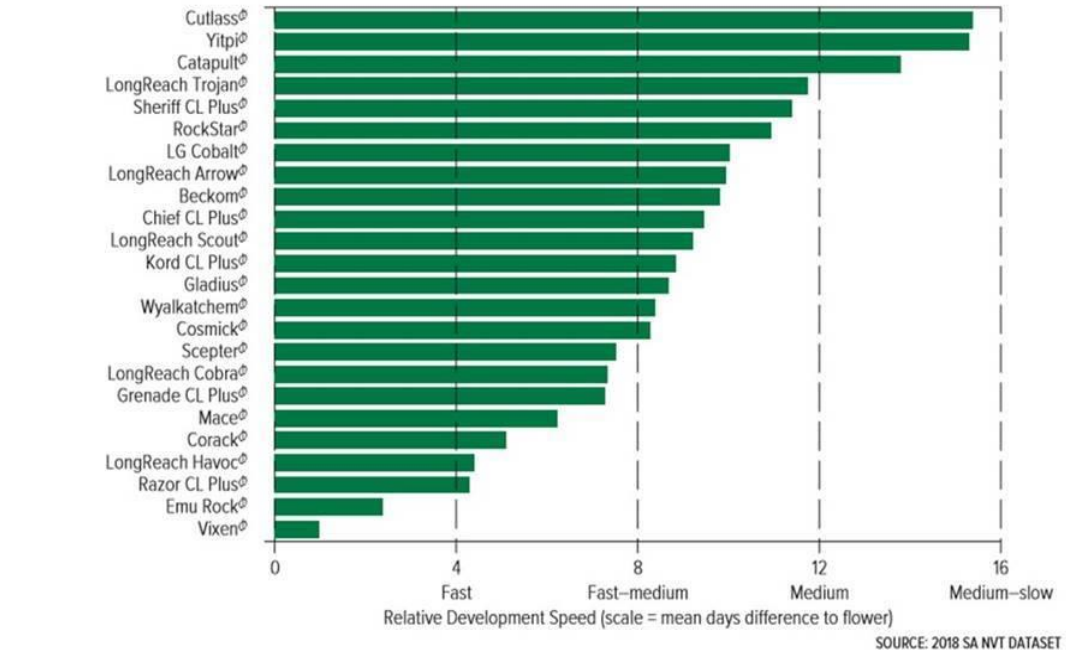

FIGURE 1 Relative speed of development of selected wheat varieties sown during

- I would choose the components based on their yield in trials compared to the industry standards like Mace and Scepter – so I jumped on the GRDC's NVTonline (https://app.nvtonline.com.au/lty/table/wheat/) site to find relevant variety comparison data. For our area, I chose the GRDC's NVT trials which are similar in rainfall and soil type to Pinery and then downloaded the yields – figures are % of site means for each year (the higher value the better).
- It is easy to choose an early flowering option – Vixen
- It is also easy to pick a midseason variety (Scepter)
- It is harder to choose a later variety – Yitpi is outclassed. Apart from that you could consider Sherriff , Cutlass (APW only), Trojan (APW only) , Catapult and Rockstar – of these, Rockstar looks to be the highest yielding of the later varieties for our area (but only 2 years of data)

Variety	Group	Trials	2015	2016	2017	2018	2019
		Mean Yield	3.08 t/ha	5.45 t/ha	4.21 t/ha	2.20 t/ha	2.40 t/ha
Vixen	AH	15		110	109	110	117
RockStar	AH	6				110	115
Scepter	AH	18	112	104	111	106	111
Catapult	AH	7				106	110
LRPB Trojan	APW	18	102	110	106	104	103
Sheriff CL Plus	APW	11		104		103	105
Mace	AH	18	109	97	105	100	102
Cutlass	APW	18	95	103	102	101	99
Chief CL Plus	APW	15		93	103	97	95
Yitpi	AH	18	89	96	96	97	95

- As a guide, the predicted yield of a mixture is likely to be close to the arithmetic mean of the components. In my studies, I conducted 81 experiments with mixtures of either wheat or oat varieties. In 6 cases the mixtures outperformed the arithmetic mean of the components, 72 times not different, and 3 times less than the arithmetic mean of the components. In % terms, on average, the mixtures had a 1% advantage over the mean of their components. The consensus of studies carried in other crop ecosystems was generally a small yield advantage for the mixtures or equal.
- If there is a frost event when one of the components is vulnerable, I would expect that the other 2 components will be OK and that there will be some compensation from the other components as well. If there are multiple frosts affecting more than one component, I still have the option of cutting for hay (although it will be harder to choose an ideal cutting time given the wider spread of flowering times than in a pure line).
- Note that the frequency of components in a mixture changes quickly over successive generations and not always do those varieties which yield highest in monoculture increase in frequency. So, to ensure that mixtures retain their integrity and remain fit for purpose, you probably need to reconstitute the mixture every year – this has the advantage of being able to update the components as new varieties become available. In the UK when mixtures of spring barley reached their peak commercial adoption in the 1980's , some seed producers offered a service of making custom mixtures from their catalogue of pure varieties and had large concrete mixers on hand to mix the components.

- One of the other benefits of mixtures is that they can slow the development of foliar disease if they are genetically different enough from each other. In fact, during the 1970's and 1980's, mixtures and multilines were the subject of intense research as a strategy to reduce disease severity and extend the life of disease resistance genes. The theory was that mixtures and multilines were more like a natural ecosystem (than a monoculture) and by being genetically diverse, they would slow disease spread. Since part of the crop was susceptible to disease, there was likely to be less pressure on the pathogen population to evolve to more and more virulent strains. In the wheat and oat mixtures I studied, the disease severity was indeed lower than the arithmetic mean (where mean = $(x_1+x_2…x_n)/n$) of the components and in fact was closer to the geometric mean (where mean = $nV(x_1.x_2…x_n)$). This was supported by numerous studies in other crops and cropping ecosystems. So, you could expect that mixtures strategy should perform better in situations where disease occurs than a pure line strategy. However, the debate about whether mixtures do extend the life of the resistance genes contained therein has to my knowledge never been resolved clearly.
- From the literature, three component mixtures are thought to be a more stable and predictable yield than two component mixtures, and superior for disease control. In my studies, I compared 2, 3 and 4 component mixtures for their effect on disease control and yield. Overall, I found 3 component mixtures were the better option. There did not seem much to gain from going to 4 component mixtures.
- You will get graded and paid for the lowest quality variety in the mix, so if you had a 3 way mix of AH, AH and an APW variety then you will be paid as APW. Note that the Viterra system cannot handle variety mixtures and hence the PBR will not be paid correctly. However, I believe Grainflow can record mixtures and so you can estimate the PBR shares (eg 1/3:1/3:1/3 in a three-component mixture). Love to hear from some of the plant breeders as to how best to deal with this strategy from an EPR point of view and ensuring equitable returns to the breeders.
- So, if I had seed of all the varieties in the table above to choose from, I would make a mixture of Vixen, Scepter and Rockstar (all AH quality)
- However, I don't have any Rockstar yet so my 2020 mixtures will be Vixen, Scepter and Trojan because that is what I have seed of (so APW quality grading)

I will leave the decision to you as to whether variety mixtures have a role on your farm and good luck for 2020! We probably won't get any frosts…….
Cheers

Andy Barr

CROP SCIENCE SOCIETY
OF SA INCORPORATED

Antiviral potential of selenium and self-heal

G. H. Lyons PhD, March 2020

School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Urrbrae, South Australia 5064, Australia

1 INTRODUCTION

Viruses comprising RNA cause a range of conditions in humans, from relatively mild (e.g. rhinoviruses causing *the common cold*) to very severe (e.g. Ebola haemorrhagic disease, with a case fatality of around 60%). Influenza, polio, rabies, measles, hepatitis A and C and coronavirus disease are all caused by RNA viruses. In livestock, RNA virus diseases include bird flu, equine flu, swine flu and foot and mouth disease.

The *Spanish Flu* pandemic of 1918-19 and the *Black Death* of the 14th century in Europe have been the most devastating disease events in history, each causing an estimated 50 million fatalities (Scott and Duncan, 2001; Benedictow, 2005; Chandra and Kassens-Noor, 2014). Some researchers regard the rapid spread and symptoms of the Black Death to be more indicative of an Ebola-type haemorrhagic RNA viral disease than the plague bacillus (Scott and Duncan, 2001). The Black Death behaved in a way plague simply cannot. It raced across the Alps and through Northern Europe at temperatures too cold for fleas to hatch and swept from Marseilles to Paris at 4km/day, far faster than a rat could travel. Iceland had no rats at all, yet the Black Death was reported there too (Scott and Duncan, 2001). An alternative case has been made for influenza virus similar to that of 1918-19 as the cause of both the Black Death and the Justinian Plague (540-543 AD). The haemolytic and cytokine storm symptoms occurring in many individuals during the 1918-19 H1N1 flu pandemic often led to the appearance of skin eruptions and bullae (lymphatic swellings), also seen in bubonic plague (Altschuler and Kariuki, 2009).

RNA viruses are more unstable and subject to higher mutation rates than DNA viruses. This makes control of the diseases they cause more difficult (Domingo, 1997). Pharmaceutical antiviral drugs, e.g. *Relenza* and *Tamiflu* are quite ineffective against most RNA viruses (Jefferson et al, 2014). This, combined with the large number of incident cases of RNA viral diseases globally, along with their frequent severity in terms of morbidity and mortality, underline the imperative to identify effective alternative antiviral agents. Even a modest therapeutic benefit would translate to a large improvement globally.

2 SELF-HEAL and SELENIUM v RNA VIRUSES

Numerous plants and their active components exhibit antiviral activity (Mishra et al, 2013), and one of the most effective is “self-heal” (*Prunella vulgaris*), a mint-family herb. Evidence includes activity against HIV (Kageyama et al, 2000; Oh et al, 2011), lentivirus (Brindley et al, 2009), ebola (Zhang et al, 2016; Yang et al, 2017) and infectious haematopoietic necrosis virus (IHNV) (Li et al, 2019). Key therapeutic components of *Prunella* include betulinic acid, prunellin, delphinidin, oleanolic acid, rosmarinic acid and ursolic acid (Anwar et al, 2018).

Low selenium (Se) (i.e. less than around 70 micrograms/l in plasma, a level common in Sub-Saharan Africa and parts of China) reduces immunocompetence and thus increases the susceptibility of the host to infection. However, and perhaps more importantly, it can also influence the genetic make-up of the viral genome. Under Se deficiency, inherently unstable RNA viruses tend to further destabilise and mutate to more virulent forms (Beck et al, 2004).

Keshan disease killed many people in Se-deficient regions of China, but has been controlled, largely with selenized salt. In most cases, a malignant cardiotropic variant of the Coxsackie B3 virus was implicated (Yang et al, 1998; Christophersen et al, 2013). Laboratory studies found that Coxsackie B3-resistant mice become susceptible to the virus under Se and vitamin E deficiency (Beck et al, 2003). Other RNA viruses exhibit increased virulence in Se deficient regions. Haemorrhagic fever with renal syndrome (HFRS) caused by hantaviruses and transmitted by rodents is a public health issue in China. A study found the incidence of HFRS in humans was around six times higher in severely Se-deficient areas and double in moderately deficient areas compared to non-deficient areas (Fang et al, 2015).

A plausible explanation for the association (shown in several studies) of low Se status and severity of HIV disease may be reduced effectiveness of cellular systems of antioxidant defence and enhanced transcription of "the AIDS gene" *HIV-1 nef*. This further depletes Se, leading to immunodeficiency (Christophersen et al, 2013 Taylor et al, 2016). A similar hypothesis has been advanced for Ebola, which appears, in common with a number of other RNA viruses, to encode selenocysteine. Biosynthesis of this protein could impose a high Se demand on the host, leading to lipid peroxidation, cell membrane breakdown and haemorrhagic symptoms (Ramanathan and Taylor, 1997). A role for Se in Ebola treatment (Lyons, 2014) is supported by Chinese researchers who treated patients in an outbreak of viral haemorrhagic fever with oral sodium selenite, which resulted in a rapid drop in mortality: after nine days of Se dosage, the death rate fell from 100% (untreated) to 37% (treated) in the very severe cases, and from 22% to zero in the less severe cases (Hou, 1997). Other studies support an antiviral role for Se and its derivatives (Wojtowicz et al, 2004; Lin et al, 2018; Spengler et al, 2019; Vasireddi et al, 2019). Organic Se forms (e.g. Se-yeast, selenomethionine, selenocysteine) may be more effective antivirals than inorganic Se (e.g. selenite, selenate) (Pan et al, 2008; Shojadoost et al, 2019).

3 CONCLUSIONS

Evidence suggests a role for both Se and *Prunella vulgaris* against RNA viruses. Why not combine them to increase the efficacy of the intervention? Research is needed to determine the most effective way to biofortify *Prunella* with Se, whether foliar or soil-applied, and what dose. It could also be naturally biofortified if grown on high-Se soils in places like Enshi, China. Studies of Se and human health have previously focused more on antioxidant, heavy metal binding, thyroid gland, anticancer and anti-heart disease effects. Judicious application of this surprising element, e.g. in combination with proven antiviral herbs, may prove to be a useful weapon against RNA viral diseases that threaten humanity.

SELECTED REFERENCES

Altschuler E and Kariuki Y 2009. Was the Justinian Plague caused by the 1918 flu virus? *Medical Hypotheses* 72: 234.

Anwar S, Pandey A, Singh M, Firdous N et al 2018. Ethno-botanical potential of *Prunella vulgaris* and human health. Scope of phytochemically unexplored medicinal plants. *ResearchGate.*

Benedictow, O. 2005. The Black Death: the greatest catastrophe ever. *History Today* 55: 42-49.

Beck M, Handy J, Levander O 2004. Host nutritional status: the neglected virulence factor. *Trends in Microbiology* 12: 417-423.

Chandra S., Kassens-Noor E 2014. The evolution of pandemic influenza: evidence from India, 1918-19. *BMC Infectious Diseases* 14: 501 doi: 10.1186/1471-2334-14-510.

Domingo E 1997. RNA virus evolution, population dynamics, and nutritional status. *Biological Trace Element Research* 56: 23-30.

Fang L-Q, Goeijenbier, Zuo S-Q, Wang L-P et al 2015. The association between hantavirus infection and selenium deficiency in mainland China. *Viruses* 7: 333-351.

Hou J-C 1997. Inhibitory effect of selenite and other antioxidants on complement-mediated tissue injury in patients with epidemic hemorrhagic fever. *Biological Trace Element Research* 56: 125-130.

Li B, Hu Y, Li J, Shi K 2019. Ursolic acid from Prunella vulgaris L. efficiently inhibits IHNV infection in vitro and in vivo. *Virus Research* 273: 197741.

Lyons GH 2014. Selenium needed for Ebola treatment. *African Journal of Food, Agriculture, Nutrition and Development* 4: 1-4.

Mishra K, Sharma N, Drishya D et al 2013. Plant derived antivirals: a potential source of drug development. *Journal of Virology and Antiviral Research* 2:2 doi: org/10.4172/2324-8955.1000109.

Oh C, Price J, Brindley M, Widrlechner M et al 2011. Inhibition of HIV-1 infection by aqueous extracts of Prunella vulgaris L. *Virology Journal* 8: 188.

Scott S, Duncan C 2001. *Biology of plagues: evidence from historical populations*. Cambridge, England: Cambridge University Press.

Shojadoost B, Kulkarni R, Yitbarek, Laursen A et al 2019. Dietary selenium supplementation enhances antiviral immunity in chickens challenged with low pathogenic avian influenza virus subtype H9N2. *Veterinary Immunology and Immunopathology* 207: 62-68.

Spengler G, Kincses A, Mosolygo T, Marc M et al 2019. Antiviral, antimicrobial and antibiofilm activity of selenoesters and selenoanhydrides. *Molecules* 24. piiE4264.

Vasireddi M, Crum A, May H, Katz D, Hilliard J 2019. A novel antiviral inhibits Zika virus infection while increasing intracellular glutathione biosynthesis in distinct cell culture models. *Antiviral Research* 161: 46-52.

Yang G, Ge K, Chen J, Chen X. 1988. Selenium-related endemic diseases and the daily selenium requirement of humans. *World Reviews in Nutrition and Diet* 55: 98-152.

CROP SCIENCE SOCIETY
OF SA INCORPORATED

Bread wheat with high salinity and sodicity tolerance

Yusuf Genc, Klaus Oldach, Julian Taylor and Graham H Lyons

Abstract

Soil salinity and sodicity are major constraints to global cereal production but breeding for tolerance has been slow. Narrow gene pools, over-emphasis on the sodium (Na^+) exclusion mechanism, little attention to osmotic stress/tissue tolerance mechanism(s) in which accumulation of inorganic ions such as Na^+ is implicated and lack of a suitable screening method have impaired progress. The aims of this study were to establish genetic variation for Na^+ concentration in modern bread wheat, compare growth responses to salinity and sodicity in low-Na^+ bread Westonia with *Nax1* and *Nax2* genes and high-Na^+ bread wheat Baart-46, and evaluate growth responses to salinity and sodicity in bread wheats with varying leaf Na^+ concentrations. The novel high-Na^+ bread wheat germplasm, MW#293, had higher grain yield under salinity and sodicity, in absolute and relative terms, than the other bread wheat entries tested. As most modern bread wheats are efficient at excluding Na^+, further reduction in plant Na^+ is unlikely to provide agronomic benefit. The salinity and sodicity tolerant germplasm MW#293 provides an opportunity for the development of future salinity/sodicity tolerant bread wheat.

Introduction

Soil salinity and sodicity severely constrain crop production in Australia and worldwide. The total global area of saline and sodic soils is estimated to be around 830 million hectares, more than 6% of the world's land and rising. Indeed, it is estimated that over 50% of global arable land will be salinized by 2050. Yield reductions of 50% in durum wheat under dryland salinity, 88% in bread wheat under high irrigation salinity and 70% under sodicity have been reported. These studies highlight the scale of lost productivity on saline and sodic soils, and the great opportunity if yield in these environments can be improved.

When cropping on saline and sodic soils, there are limited options to raise productivity, and they are complementary: (i) soil management and (ii) plant breeding. Despite the potential of the plant breeding approach, progress in breeding cereal cultivars with salinity or sodicity tolerance has been slow. This is often attributed to the genetic and physiological complexities of the salt tolerance trait, and lack of a reliable and rapid screening assay. Moreover, elite germplasm may not include genes able to confer worthwhile salt/sodicity tolerance, and introgression from wild wheat relatives and/or genetic engineering may be required for step change progress to be achieved.

An example of the use of a wild relative is the work of Richard James and his colleagues, who introgressed Na^+ exclusion genes *Nax1* and *Nax2* from the diploid bread wheat ancestor *Triticum monococcum* L. (C68-101) into durum wheat Tamaroi. *Nax1* removes Na^+ from the xylem in roots and leaf sheaths, while *Nax2* removes Na^+ from xylem in the roots only. Tamaroi with *Nax2* showed lower leaf Na^+ concentration and achieved higher grain yield under salinity and sodicity. These two genes were also transferred from durum wheat into bread wheat cv. Westonia, and subsequently shown to reduce leaf 3 Na^+ concentration. A recent saline field trial with three Westonia-*Nax2* and two Westonia-*Nax1* lines indicated, compared to Westonia, a 9% yield increase (average over two seasons) in one of the Westonia-*Nax2* lines (Westonia-*Nax2*-5924). These results are encouraging,

but not conclusive. Therefore, there is a need to verify the effects of these genes in bread wheat in controlled environment studies involving salinity and sodicity, especially as bread wheat has much greater Na^+ exclusion than durum wheat. Despite their potential for improving salinity tolerance, wild relatives and landraces of bread wheat largely remain an untapped resource. In the early 2000s salinity tolerant bread wheat germplasm lines W4909 and W4910, derived from wild relatives, were developed by Richard Wang and his colleagues. However, these germplasm lines have not been exploited in breeding programs.

Sodicity, of which high Na^+ is the key component, affects greater land area than salinity but there has been little specific research on sodicity and mechanisms of tolerance. This is unsurprising as screening for sodicity tolerance has been difficult in laboratory or glasshouse environments, which are needed to test large numbers of accessions in a relatively controlled manner. Problems with current screening methods include (i) very high pH of sodic soils, hence difficulty of separating pH effects from those of Na^+ toxicity, (ii) inability to control soil composition when sourced from field sites, and (iii) months of waiting before pH stabilizes, and thereafter the possibility of toxicity from excess salt (sodium bicarbonate) not adsorbed at cation exchange sites. A recently developed soil-based screening method, using Na^+-humate as a surrogate for sodicity, avoids these issues and enables screening of a large number of accessions. It is important to note that the Na-humate method specifically refers to the high sodium component of sodicity, and does not address physical constraints. We utilised this method in order to determine genotypic variation in Na^+ exclusion in commercial bread wheat varieties and assess its importance to sodicity and salinity tolerance.

The aims of this study were to (i) establish genetic variation for Na^+ concentration in modern bread wheat (n=98), (ii) compare growth responses to salinity and sodicity in low-Na^+ bread wheat Westonia with *Nax1* and *Nax2* genes and high-Na^+ bread wheat Baart-46, and (iii) evaluate growth responses to salinity and sodicity in bread wheats with varying leaf Na^+ concentrations.

Results/Discussion

Genetic variation in Na^+ accumulation in 100 bread wheat entries

Figure 1 shows that there is genetic variation in Na^+ exclusion, but almost all elite bread wheat entries had high Na^+ exclusion (approx. <2,000 mg Na^+ kg^{-1} DW), compared to typical durum wheat entries (approx. 15,000-30,000 mg Na^+ kg^{-1} DW). Leaf Na^+ concentrations in bread wheats varied from 50 mg kg^{-1} DW in Westonia-*Nax2* to 2,800 mg kg^{-1} DW in cv. Olympic. The only exceptions to this were two bread wheat germplasm lines (MW#451 and MW#293; approx. >15,000 mg Na^+ kg^{-1} DW) which grouped with the durum wheats. The presence of Na^+ exclusion genes *Nax1* and *Nax2* in durum wheat (*Nax1* and *Nax2* in WID902; *Nax2* in Tamaroi) was associated with much lower Na^+ concentrations (approx. 600-4,000 mg kg^{-1} DW) than in durum wheats lacking these genes.

Effects of Nax1 and Nax2 genes on salinity and sodicity tolerance in low-Na^+ bread wheat cv. Westonia as compared to high-sodium bread wheat cv. Baart-46

Penultimate leaf Na^+ concentrations were higher under sodicity than salinity (**Figure 2).** Baart-46 maintained higher Na^+ concentrations than Westonia and Nax lines under both stresses. As compared to Westonia, the presence of *Nax1* and *Nax2* genes was associated with reduced Na^+ concentrations. Reductions were similar for both genes, and became more pronounced at higher rates of salinity and sodicity, reaching maxima of 72-82% and 32-34% reductions at 8 g kg^{-1} Na^+-humate and 100 mM NaCl, respectively. However, reduced Na^+ concentrations in the Westonia Nax lines were not accompanied by higher grain yield, with small grain yield increases observed only under moderate salinity and low

sodicity. At these salinity and sodicity rates, despite much higher Na^+ concentrations than Westonia and Nax lines, cv. Baart-46 was similar or higher for grain yield.

Our results confirm that Westonia-*Nax1* and Westonia-*Nax2* lines were lower in leaf Na^+ concentration compared to Westonia, and showed slightly higher but non-significant grain yield increase at moderate salinity (50 mM NaCl) and low sodicity (2 g kg^{-1} Na^+-humate). However, compared to high-Na^+ bread wheat Baart-46, Na^+ concentrations of Westonia and Nax lines were low, and hence small differences in Na^+ concentration between Westonia and the Nax lines are unlikely to make a difference to grain yield. This supposition is supported by two lines of evidence: Baart-46 had much higher Na^+ concentration but yielded higher than the three Westonia lines at all levels of salinity and sodicity. Secondly, in a saline field trial, only one Westonia-*Nax2* line (5924) yielded higher (11%) than Westonia, while the other four Westonia-Nax lines were, on average, no different to Westonia. The results indicate that transferring *Nax1* and *Nax2* genes into an already efficient Na^+ excluding bread wheat confers little, if any, improvement in overall salinity tolerance. Unlike low-Na^+ bread wheat, when the *Nax2* gene was introduced into high-Na^+ durum wheat cv. Tamaroi, a significant yield increase was reported under salinity in the field and under sodicity in the growth room. The differences between the Na^+ excluding abilities of bread and durum wheats are attributed to modern bread wheats possessing homologs of the Na^+ exclusion genes *Nax1* and *Nax2* and/or other Na^+ exclusion genes while durum wheats are thought to lack such genes. Hence, the introduction of Nax type genes is more useful in durum wheat backgrounds.

Effects of a wide range of Na^+ exclusion on salinity and sodicity tolerance in 20 bread wheats, three durum wheats and a barley entry

Salinity and sodicity increased leaf Na^+ concentrations in all entries, and concentrations were higher under sodicity than salinity (**Table 1**). Amongst the commercial wheats, older cultivars such as Federation and Baart-46 had higher Na^+ concentrations (approx. 430-460 and 1,700-1,800 mg kg^{-1} DW under salinity and sodicity) than modern cultivars (approx. <400 and 1,200 mg kg^{-1} DW under salinity and sodicity) (**Table 1)**. However, none of the cultivars had Na^+ concentrations as high as the two novel germplasm lines (MW#451 and MW#293) derived from wild relatives of bread wheat (*Thinopyrum junceum and Aegilops speltoides)*; (averages of these two lines; approx. 5,600 and 13,000 mg Na^+ kg^{-1} DW under salinity and sodicity, respectively) (**Table 1**). Barley entry Clipper had Na^+ concentrations (approx. 7,000 and 17,000 mg kg^{-1} DW under salinity and sodicity) as high as those in high- Na^+ wheat germplasm lines MW#293 and MW#451, while durum wheats Yawa and Tamaroi had overall the highest Na^+ concentrations (approx. 6,700 and 11,400 under salinity; 20,600 and 32,700 mg kg^{-1} DW under sodicity, respectively).

Bread wheat entries varied in grain yield under control, sodicity and salinity (3-, 4- and 5-fold, respectively; **Figure 3**). Axe produced the lowest, while germplasm line MW#293 produced the highest grain yield under all conditions and doubled the grain yield of almost all other entries under salinity and sodicity (**Figure 3**). Depending on wheat entries, tolerance (relative grain yield %) was higher, lower or similar between salinity and sodicity (**Figure 3**). The most noteworthy effects were the higher sodicity tolerance in Tamaroi-*Nax2* compared to Tamaroi, and the highest salinity and sodicity tolerance in MW#293 (**Figure 3)**.

Sodium exclusion in bread wheat and its relationship with salinity and sodicity tolerance as measured by a novel screening method

Our results, along with other studies, demonstrate that whilst there is genetic variation for Na^+ concentration in modern bread wheat, most wheats contain relatively low Na^+ concentrations. In modern bread wheat we found no correlation between leaf Na^+ concentration and either salinity or sodicity tolerance based on grain yield (n=18). In fact, wheat germplasm MW#293 carrying alien introgressions achieved the highest salinity tolerance despite having a 14-fold higher Na^+ concentration (6,044 mg kg^{-1} DW) than the highest of the naturally occurring bread wheats (cv. Federation, 425 mg kg^{-1} DW). Similarly, under sodicity MW#293 had a 7-fold higher Na^+ concentration (12,939 mg kg^{-1} DW) than the second highest bread wheat cv. Federation (1,651mg kg^{-1} DW), and still had the highest sodicity tolerance. Despite the prevailing opinion that low Na^+ confers tolerance, our results in and other studies in wheat, barley and maize show that low Na^+ concentration is not necessarily associated with salinity tolerance. This suggests that additional mechanisms (tissue tolerance/osmotic adjustment) need to be considered in order to breed salinity tolerant bread wheat.

A novel wheat germplasm (MW#293) for development of future salinity/sodicity tolerant bread wheat

MW#293 was derived from an earlier bread wheat germplasm line (W4909) developed by Richard Wang and his colleagues in USA. W4909 is a product of three species [*Triticum aestivum cv.* Chinese Spring*), Aegilops speltoides* and *Thinopyrum junceum* (sea wheatgrass)], and its ability to accumulate very high Na^+ sodium has been demonstrated in independent studies. However, its salt tolerance is debatable as studies so far have produced variable results. In addition, the potential of high Na^+ as a source of osmotic adjustment/ tissue tolerance in a widely adapted and high yielding bread wheat has not been realized.

To introduce salt tolerance gene(s) of W4909 into a commercial bread wheat, we made a cross between a popular Australian bread wheat cv. Mace and W4909, and developed a doubled-haploid population (over 200 lines). As the population segregated for maturity and height markedly, a sub-selection of this population (n=18), agronomically similar to commercial lines, was grown under control and salinity. Of these 18 lines, MW#293 had the highest grain yield under both control and salinity, and doubled the grain yield of Mace under salinity despite having an 86-fold higher leaf Na^+. When tested with 18 commercial wheats in (which included Kharchia 65-one of the most sodicity- and salinity-tolerant landraces), MW#293 produced the highest grain yield under control, salinity and sodicity, and its grain yield under salinity was three times higher despite 35-100-fold higher leaf Na^+ concentrations (under sodicity and salinity) than cv. Mace (**Table 1; Figure 4**). Mujeed-Kazi and colleagues make the important point that breeding wheat solely for salinity tolerance at the cost of yield loss in nonsaline soils is unsuitable for farmers: “Breeders need to develop cultivars with high yield potential under both stress and nonstress conditions”, in other words *vigorous* cultivars. In a subsequent experiment, MW#293 recorded 200-fold higher leaf Na^+ concentration and 2-fold higher grain yield than cv. Mace. MW#293 also had the highest growth rates under salinity and sodicity. These data suggest that MW#293 may have the ability to efficiently assimilate and sequester Na^+ levels that can support high growth rates. To our knowledge, such high Na^+ accumulation together with high grain yield/growth rate in bread wheat has not been previously reported. This represents a new paradigm in breeding for salinity tolerance.

Conclusions

- Despite much higher leaf Na^+ concentration, bread wheat germplasm MW#293 had higher grain yield under salinity and sodicity, in absolute and relative terms, than the other bread wheat entries tested.
- Despite a 10-14 fold variation in leaf Na^+ concentration in modern bread wheats, there were no correlations between leaf Na^+ concentration and either salinity or sodicity tolerance, thus demonstrating the limits of using leaf Na^+ concentration alone as a selection parameter for salinity/sodicity tolerance.
- As modern bread wheats have an excellent Na^+ exclusion ability, further investment in the Na^+ exclusion mechanism is unlikely to improve sodicity/salinity tolerance significantly. Future efforts should focus on osmotic adjustment/tissue tolerance mechanisms.
- Genome-wide association mapping revealed novel genes associated with high Na^+ accumulation, which may be involved in osmotic adjustment/tissue tolerance.
- The salinity and sodicity tolerant germplasm MW#293 provides an opportunity for the development of future salinity/sodicity tolerant bread wheat.

Led by SARDI, this work was supported by the University of Adelaide, the University's Waite Research Institute and The Yitpi Foundation. It was dedicated to the late Professor Tony Rathjen (1940-2014) of The University of Adelaide, a revered plant breeder and lecturer whose legacy includes more than 25 wheat varieties and hundreds of influential agricultural scientists. Amongst many achievements, Tony made a significant contribution to research and breeding in the fields of salinity and sodicity. The full version of this article can be accessed at https://www.frontiersin.org/articles/10.3389/fpls.2019.01280/full.

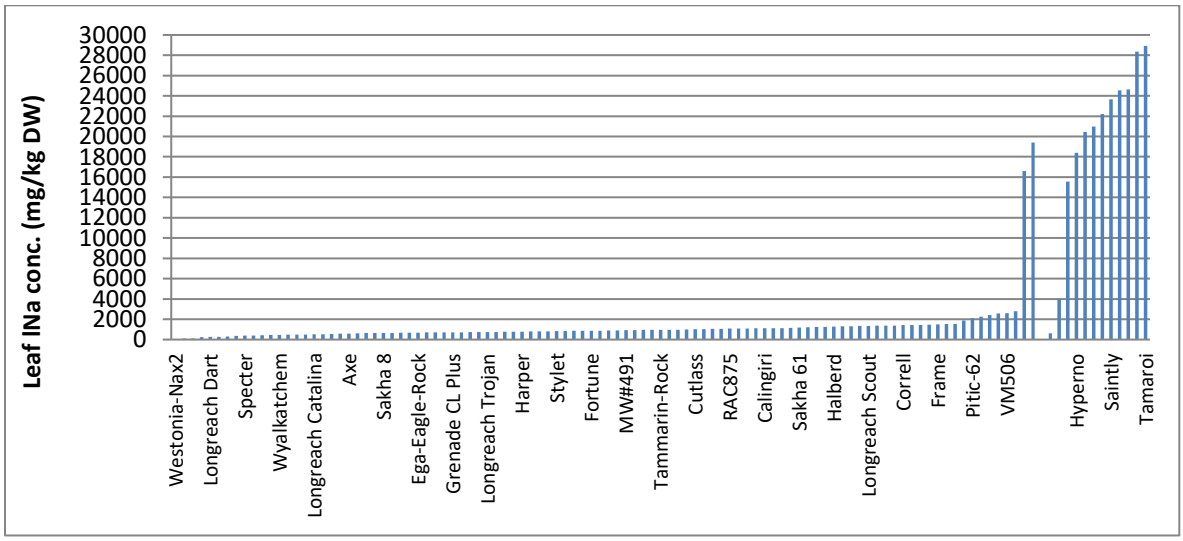

Figure 1 Penultimate leaf Na^+ concentrations (mg/kg DW) at heading in 100 bread wheat entries and 12 durum wheat entries grown under sodicity.

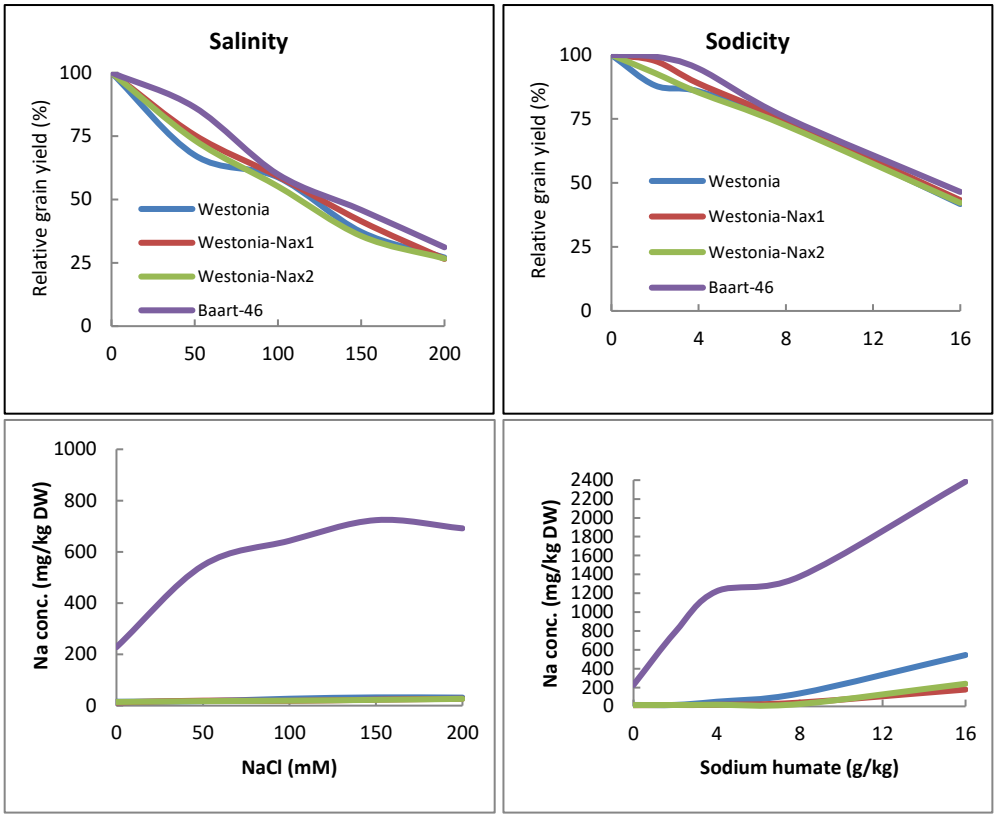

Figure 2 Relative grain yield (%) (%) (salinity or sodicity tolerance), and penultimate leaf Na^+ concentrations at heading in wheat cv. Westonia, Westonia-*Nax1*, Westonia-*Nax2* and Baart-46 under different levels of salinity (left panels) and sodicity applied as Na^+ humate (right panels).

Longreach Cobra	21	162	30
Westonia	16	232	31
Krichauff	17	325	64
Mace	24	365	63
Wyalkatchem	24	443	100
Axe	46	601	133
Halberd	16	606	126
Beckom	20	620	114
Yitpi	23	622	174
Condor	13	658	106
Kharchia-65	56	705	165
AGT Katana	18	766	125
Drysdale	162	847	318
Pitic-62	22	976	351
Correll	39	1053	151
Hartog	274	1213	383
Federation	178	1571	425
Baart-46	279	1651	417
MW#293	884	12939	6045
MW#451	1216	13063	5187
Tamaroi_Nax2	317	4197	1853
Yawa (WID 803)	1922	19339	5949
Tamaroi	2510	29025	10228
Clipper	3099	15128	6042

Table 1 Penultimate leaf Na concentrations (mg/kg DW) at heading in 20 bread wheat at heading in 20 bread wheat entries, three durum entries and one barley entry.

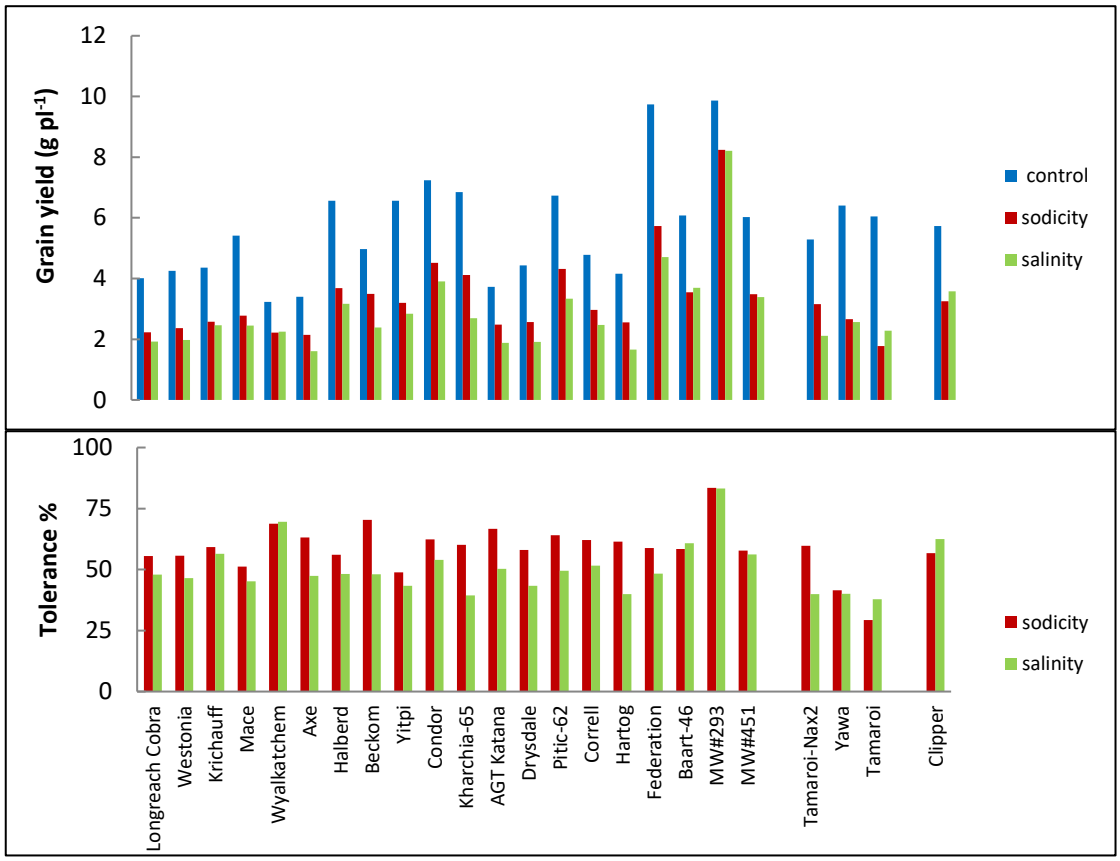

Figure 3 Grain yield, and tolerance (grain yield under sodicity or salinity as a percentage of grain yield under control) of 20 bread wheat entries (*Triticum aestivum* L.), three durum wheat entries (*Triticum turgidum* subsp durum cv. Tamaroi, Tamaroi-*Nax2* and Yawa) and one barley entry (*Hordeum vulgare* L. cv. Clipper)

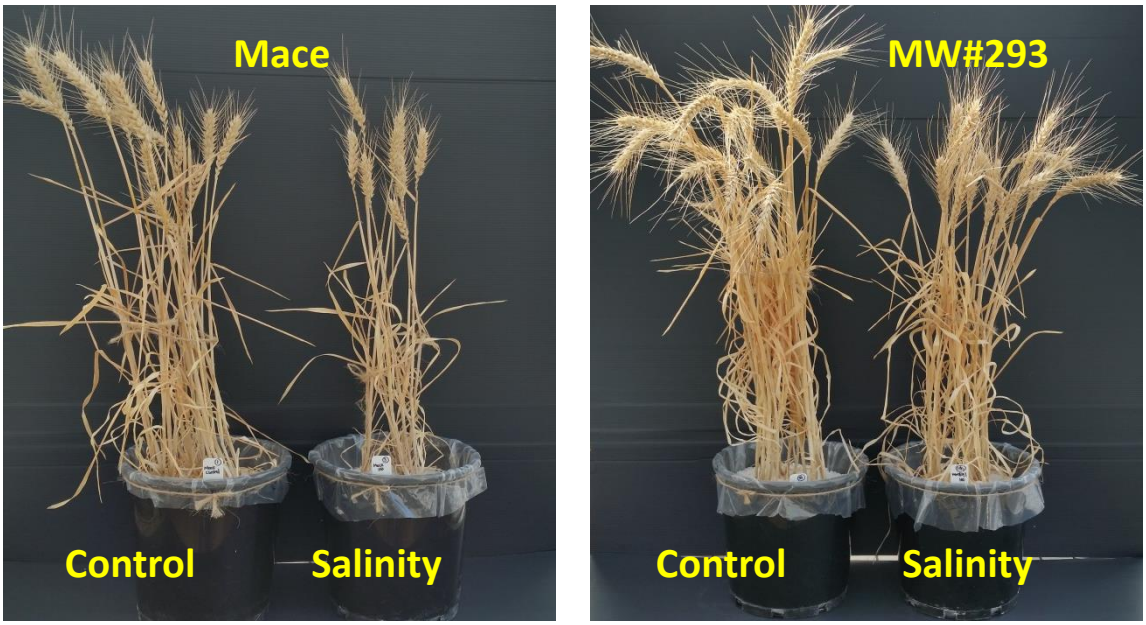

Figure 4 Representative pots of bread wheat (*Triticum aestivum)* cv. Mace and doubled-haploid line MW#293 grown under control and salinity (100 mM NaCl).