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Project Type	

FINAL REPORT 2023

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PROJECT TITLE	
Evaluating super high oleic acid safflower in sodic and saline soils	

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PRINCIPAL INVESTIGATOR			
Title:	**First Name:**		**Surname:**
Dr	Rhiannon		Schilling
Organisation:	The University of Adelaide		
Mailing address:	Level 3, Rundle Mall Plaza, 50 Rundle Mall, Adelaide SA 5000		
Telephone:		**Email:**	
Mobile:			

ADMINISTRATION CONTACT DETAILS			
Title:	**First Name:**		**Surname:**
Ms	Michelle		Whittle
Organisation:	The University of Adelaide		
Mailing address:	Level 3, Rundle Mall Plaza, 50 Rundle Mall, Adelaide SA 5000		
Telephone:		**Email:**	
Mobile:			

PROJECT REPORT:

Executive Summary

The SA Grain Industry Blueprint highlighted the need for a tenfold increase in the production of specialty oils in SA from around 2,000 tonnes to 20,000 tonnes. The genetically modified (GM) super high oleic acid (SHO) safflower (*Carthamus tinctorius* L.) developed by CSIRO and GRDC and licensed by GO Resources is a non-canola oilseed alternative with consistent oil quality that has the potential to be of high value to SA grain growers. This SAGIT funded project evaluated the role of SHO safflower for use in saline soils (Aim 1) and conducted a controlled greenhouse experiment to assess the level of tolerance of SHO safflower lines to sodic soils (Aim 2). The key findings for this project were:

Aim 1

- The SHO safflower germinated, established biomass and grain yield in both the low and high salinity (2022 results) but care is needed as a failed safflower crop can occur if the saline soil has inadequate soil moisture (2021 results).
- The current E40-R commercially available line of the SHO safflower had variable shoot biomass in the high salinity site. There were large visual biomass differences amongst the SHO safflower lines with the advanced breeding lines showing high early vigour in the saline soils.
- Shoot tissue concentrations varied between low and high salinity areas with sodium concentrations increasing in safflower plants in the high salinity compared to the low salinity.
- Higher grain yield occurred in the high salinity trial compared to the low salinity trial and this is likely due to the safflower accessing legacy nutrients from previous fertiliser applied at seeding for cereal crops that have failed to grow in this high salinity site. There was no difference in oil content between the low and high salinity safflower.

Aim 2

- Variation in tolerance to sodicity (shoot biomass in sodic relative to control soil) in the safflowers ranged from 41-60% and was less than both canola (62%) and wheat (67%).
- In all crop types, shoot sodium and boron concentrations significantly increased and shoot calcium and magnesium concentrations significantly decreased in the sodic soil compared to non-sodic control.
- Two safflower lines (G-Trt-2 and G-Trt-3) had significantly higher shoot boron concentrations than all other lines. These two lines with high boron concentrations were the least tolerant to the sodic soil. This suggests that genetic variation for boron accumulation could be selected by safflower breeders.

Project objectives

Safflower has the potential to be a valuable oilseed crop for South Australian growers. With the recent lifting of the genetically modified (GM) mortarium (except on KI), SA growers now have choice to grow commercially available super high oleic acid (SHO) safflower varieties. There has been increasing observational evidence from growers in NSW and Victoria that suggests safflowers are tolerant to sodic and saline soil types. However, to date, there had been limited trials of safflower in SA, particularly in paddocks with salinity, and evaluation of the new SHO safflower lines had not yet occurred in SA. The level of tolerance of safflowers (including SHO lines) to sodicity (high salinity, high boron, high aluminium and high pH) had also not yet been determined.

The two aims of this project were:

1. To evaluate and demonstrate the role of safflower (including SHO lines) in saline soils.
2. To conduct a pilot experiment to determine the level of tolerance of safflower lines to sodic soils.

Overall Performance

The project aims were successfully achieved with a field trial in a low and high salinity area of a paddock completed at Coomandook in 2022 (and 2021 – see below) and a controlled greenhouse experiment assessing different SHO safflower lines with a comparison to wheat and canola completed using the high-throughput phenotyping facility at The Plant Accelerator.

The personnel involved in this project included:
Dr Rhiannon Schilling, SARDI (lead CI)
SARDI Waite Agronomy team (assisted with field trial)
Mr David Hudson, GO Resources (assisted with seed source and technical input)
Dr Chris Brien, The Plant Accelerator
Dr Bettina Berger, The Plant Accelerator
Dr Nathaniel Jewell, The Plant Accelerator
Mr Tim Freak, Grower (field trial host)

Difficulties encountered
The safflower field trial at Coomandook was sown in a low and high salinity area in 2021. However, this trial was abandoned due to a very dry start to the growing season impacting on safflower establishment in the saline soil. The SARDI Waite Agronomy team repeated the field trial in 2022 in a high rainfall growing season and useful data was obtained for SA growers and SAGIT. Two grain quality samples were impacted during transport to Victoria for oil quality testing, but all other samples arrived intact and were measured. GO Resources provided access to seeds and assistance with the oil quality testing for safflower.

KEY PERFORMANCE INDICATORS (KPI)

KPI	Achieved	If not achieved, please state reason.
1. Complete a Smarthouse experiment evaluating the growth rates and water use of safflower cultivars in non-sodic and sodic soil.	Yes ☒ No ☐	N/A
2. Complete a field trial of safflower in non-saline and saline paddock.	Yes ☒ No ☐	N/A
3. Complete a final report of the project findings.	Yes ☒ No ☐	N/A

TECHNICAL INFORMATION

Aim 1

A SHO safflower field trial in both low and high salinity areas of the same paddock was sown in 2021 but was abandoned due to dry conditions (Figure 1).

Figure 1 - Low salt safflower trial at Coomandook SA in 2021 showing the stressed plants due to lack of rainfall.

This trial was repeated in the subsequent year by the SARDI Waite Agronomy team with both the low and high salinity areas sown on the 16th of June 2022. The 2022 trial was a double resolvable row-column design with 4 replicates in the low salinity site and 3 replicates per line in the high salinity site. Due to limited seed of some advanced breeding lines, there were 10 safflower lines tested in the low salinity site and 7 lines in the high salinity site. Seeding occurred shortly after a rain event and this helped with ensuring plant establishment in the saline soil.

Soil samples were collected from both the low and high salinity site at 0-5, 5-10 and 5-15 cm. The texture was measured (sandy loam) and the N, P, Ca, Mg, K, Na, B and Cl concentrations were determined as well as ECe and pH. Results of the ECe, Chloride and B concentrations are presented in Figure 2 and show that the sites were affected by salinity with high chloride and relatively low boron levels (not sodic).

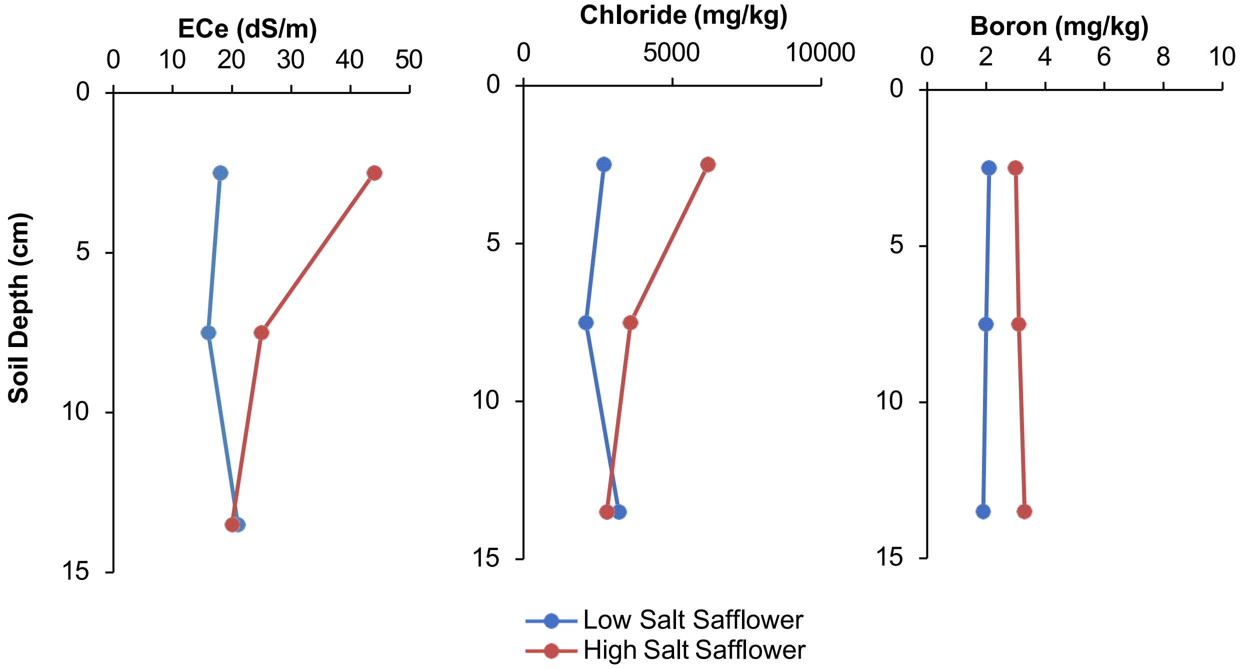


Figure 2 - Soil EC (dS/m), chloride concentration (mg/kg) and boron (mg/kg) at 0-5, 5-10 and 10-15 cm at Coomandook for the low salt (blue) and high salt (red) sites.

By the 30th of June, plants were at the 2nd leaf stage and plant establishment counts were conducted on the 2nd of August to ensure all plants were recorded following often late emergence from saline soils with 2 rows x 0.5 m of plants counted in each plot. Plant establishment showed that the plots established to equivalent levels in the low and high salinity for each line (no effect of salinity) with lower establishment in lines E40-R, SH0181, SH0441, SHO557 compared to the advanced breeding lines (X3242-2-1, X3242-4-1, X3290-1-2-1, X3403-6-1-1, X3403-7-3-1-1 and AGR101 F1) (Figure 3 a,b). Visual vigour scores were recorded (good, average and poor) in Figure 3c with the X3242-2-1, X3242-4-1, X3290-1-2-1, X3403-6-1-1, X3403-7-3-1-1 lines showing the highest level of plant vigour under both high and low salinity (Figure 3a).

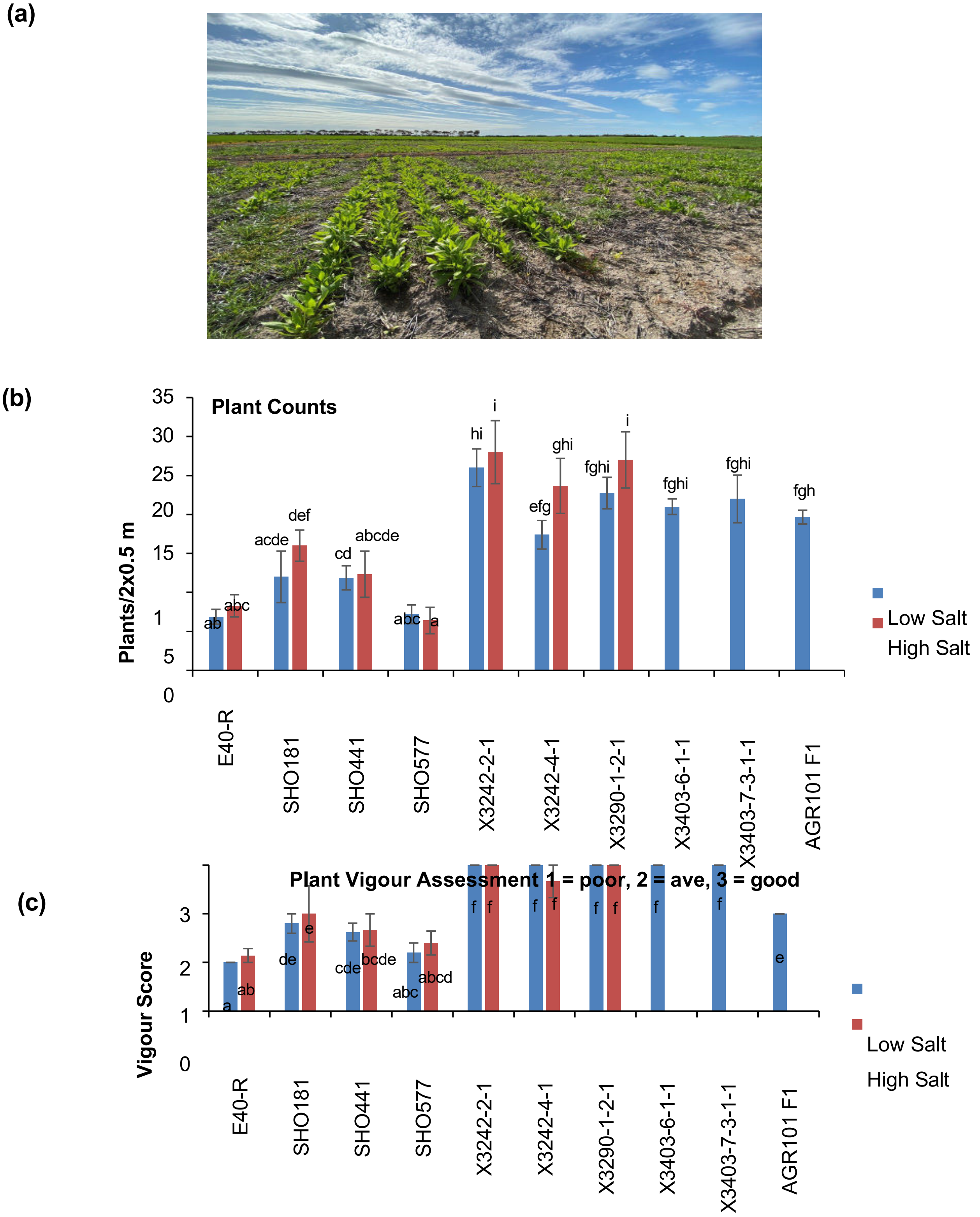


Figure 3 - The variation in (a) early vigour visible on the 13th of September 2022 among the SHO safflower varieties in the low and high salt site. (b) the plant establishment counts for both the low and high salt site and a visual plant vigour assessment (1 = poor, 2 = average, 3 = good) for each line tested in the low and high salt site on the 2nd of August 2022. Values presented as mean ± sem with significant differences indicated with different letters ($p \leq 0.001$).

Shoot biomass was collected from each plot on the 13th of September 2022 and dry weights recorded (Figure 4). The high salinity site tended to have higher biomass than the low salinity site (SHO441, X3242-2-1) or no difference in biomass between sites (E40-R, SHO181, SHO577, X3242-4-1, X3290-1-2-1). The higher biomass in the high salinity site (SHO441, X3242-2-1) is likely due to the safflower accessing legacy nutrients from previous fertiliser from seeding cereal crops that have failed to grow in this high salinity site.

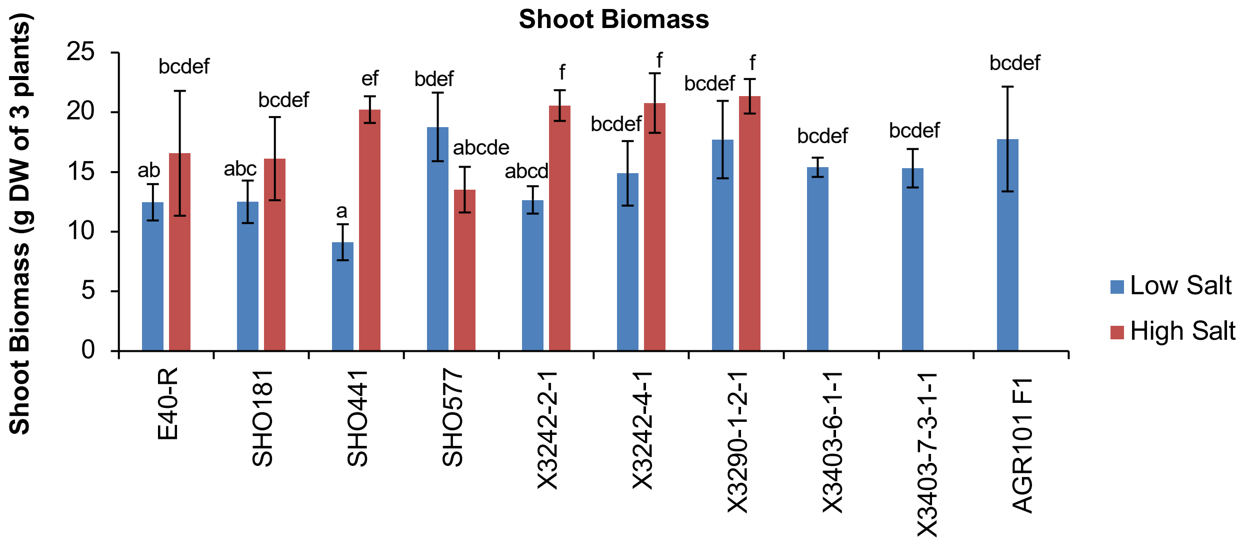
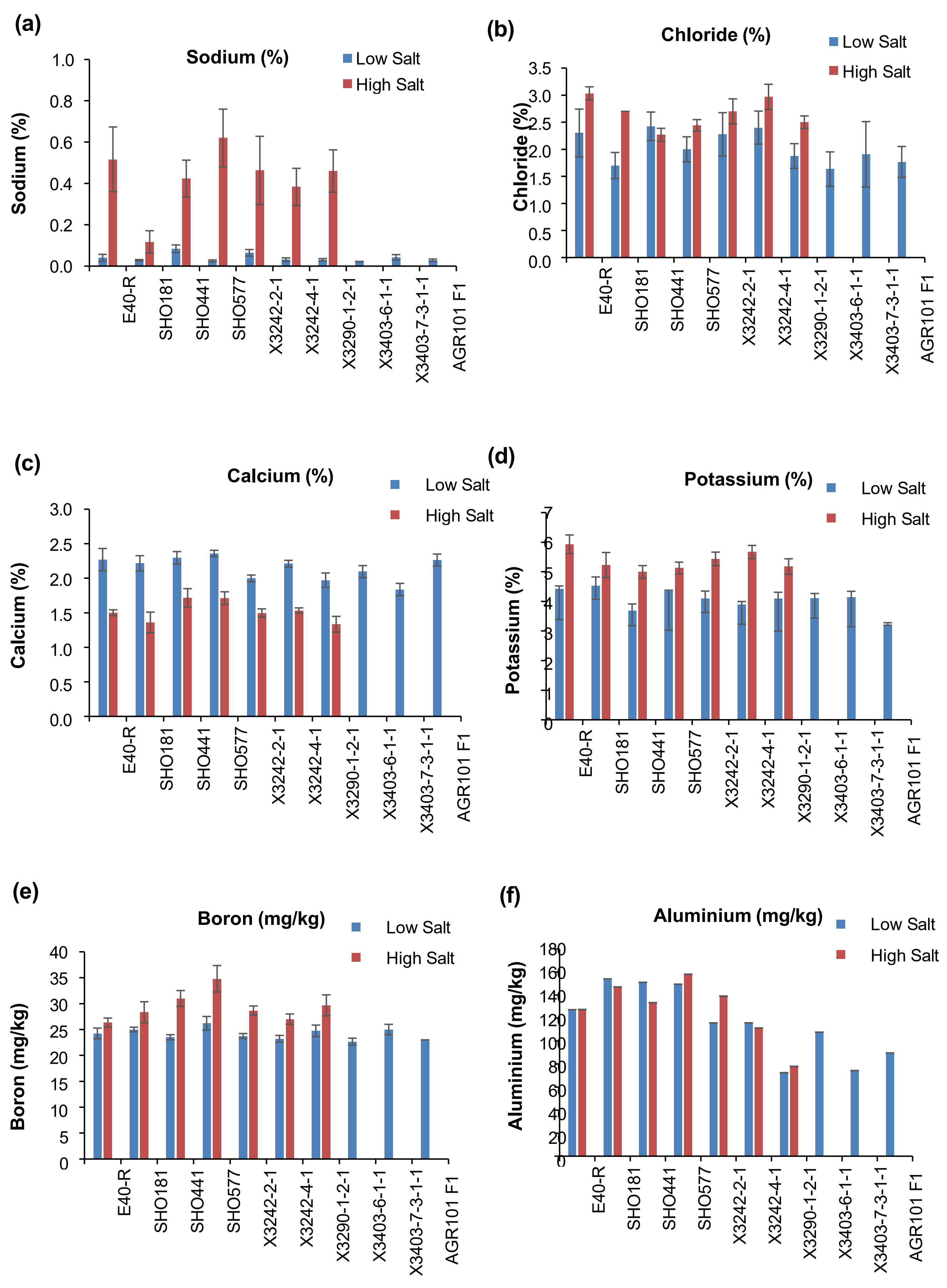


Figure 4 - The shoot biomass at branching of 3 plants per plot for each line in the low and high salt site on the 13th September 2022. Values presented as mean ± sem with significant differences indicated with different letters ($p \leq 0.001$).

Whole shoot tissue samples from each plot were tested by Eurofins APAL for ICP analysis (Figure 5 a-l). Findings show shoot sodium and potassium concentrations but not chloride increased in the SHO safflower lines in the high salinity site compared to the low salinity site (Figure 5a, b). This confirms the presence of higher sodium at this location in the paddock and suggests that safflower may be a good excluder of sodium but not chloride (higher %). A significant increase in molybdenum (all lines), boron (some lines) and potassium (some lines) were measured in the safflower lines under the high salinity compared to the low salinity site (Figure 5 d, j). A significant decrease in shoot calcium % was measured in the SHO safflower lines in the high salinity site compared to the low salinity site. This suggests that the safflower lines may benefit from increased calcium applications in saline sites, however, there is currently very limited information available on the critical nutrient levels for SHO safflower lines to determine deficiency ranges. All significant ICP values are presented in Attachment 1.

The plants progressed well to maturity (Figure 6a). The grain yield of each plot was recorded in January and showed higher yields in the high salinity area compared to the low salinity area. The differences in biomass did not necessarilyl translate to differences in grain yield with nearly all lines (except X3290-1-1-2) having higher grain yield in the high salinity area compared to the low salinity area. As discussed above, this is likely due to the plants in the high salinity area accessing nitrogen from past failed crops. The oil content (%) was measured by GO Resources through David Hudson and indicate no significant differences in oil content between the lines or salinity areas with values around 35%.


(a)
Sodium (%)
Low Salt
High Salt
Sodium (%)
1.0
0.8
0.6
0.4
0.2
0.0
E40-R
SHO181
SHO441
SHO577
X3242-2-1
X3242-4-1
X3290-1-2-1
X3403-6-1-1
X3403-7-3-1-1
AGR101 F1
(b)
Chloride (%)
Low Salt
High Salt
Chloride (%)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(c)
Calcium (%)
Low Salt
High Salt
Calcium (%)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(d)
Potassium (%)
Low Salt
High Salt
Potassium (%)
7
6
5
4
3
2
1
0
(e)
Boron (mg/kg)
Low Salt
High Salt
Boron (mg/kg)
40
35
30
25
20
15
10
5
0
(f)
Aluminium (mg/kg)
Low Salt
High Salt
Aluminium (mg/kg)
180
160
140
120
100
80
60
40
20
0

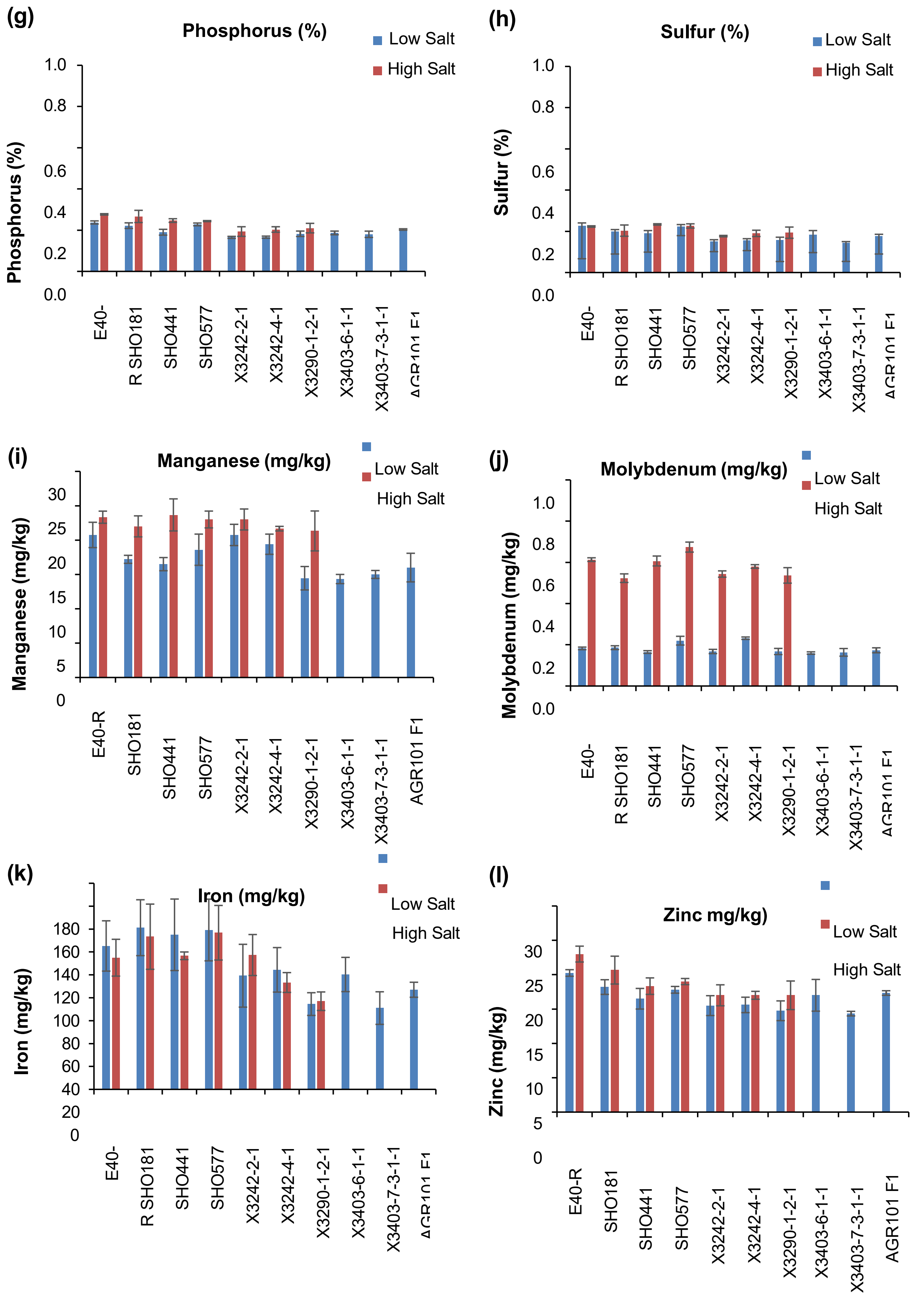


Figure 5 - The ICP analysis results for the whole shoot at branching for (a) sodium, (b) chloride, (c) calcium, (d) potassium, (e) boron, (f) aluminium, (g) phosphorus, (h) sulfur, (I) manganese, (j) molybdenum, (k) iron and (l) zinc at low (blue) and high (red) salinity areas in Coomandook in 2022. Significant differences are shown in Attachment 1.

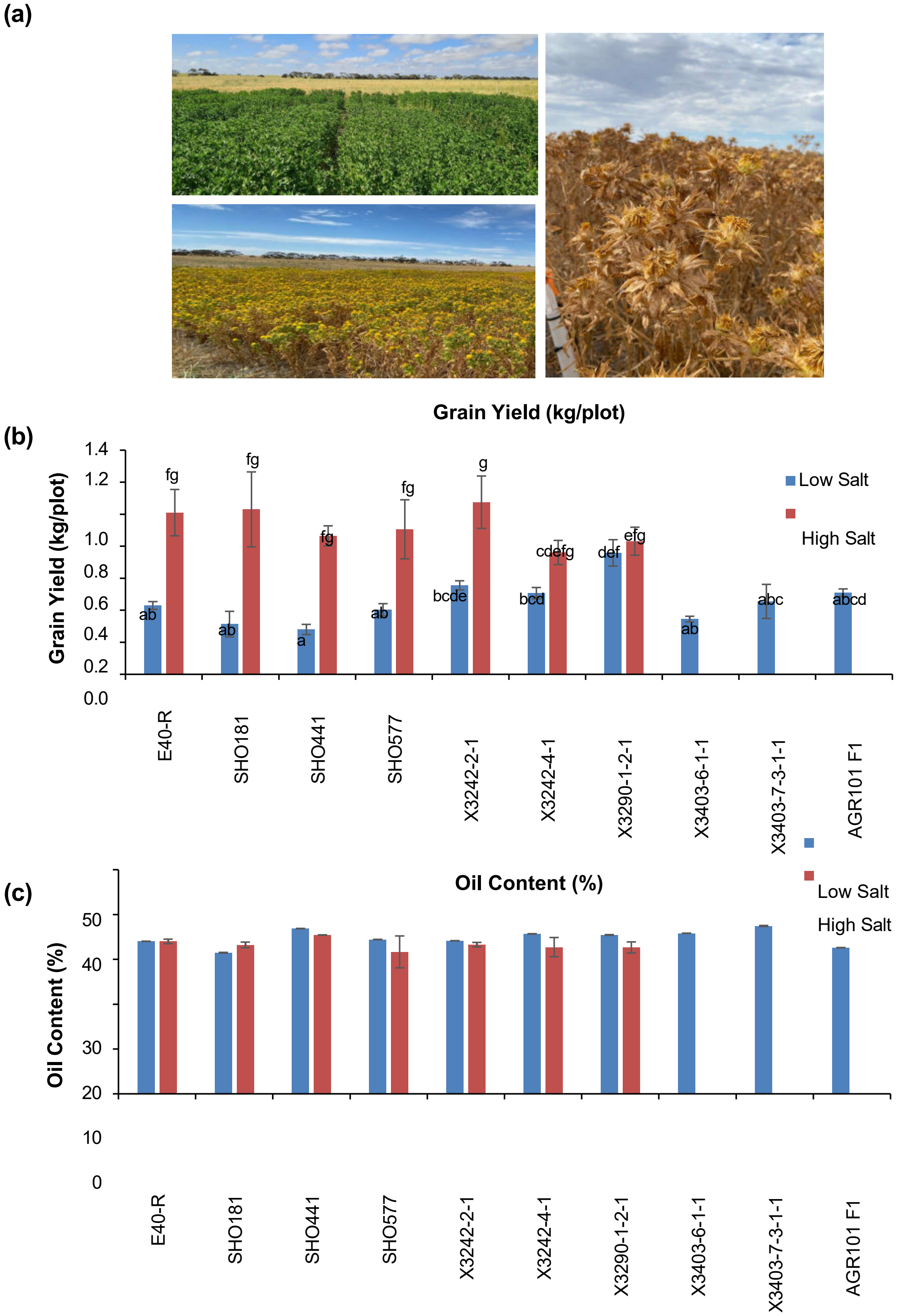


Figure 6 - The safflower (a) progressed well in the 2022 season with both high biomass and large filling capitula with (b) grain yield (kg/plot) and (c) oil content (%) for the SHO safflower in low (blue) and high (red) salinity areas at Coomandook in 2022. Values presented as mean ± sem with significant differences indicated with different letters ($p \leq 0.001$). Please note SHO 441 and X3242-4-1 in oil content only have 2 replicates due to grain sample loss in transport and there was no significant difference between oil content of lines or sites.

Aim 2 – Smarthouse Experiment

A high-throughput Smarthouse phenotyping experiment was conducted in The Plant Accelerator to evaluate the shoot growth rates, water use and shoot nutrient concentrations of 8× SHO safflower lines, 1× canola (cv. Binito) and 1× wheat (cv. Mace) in a non-sodic (control) and alkaline, sodic soil with 6 replicate pots per treatment (120 pots) (see Figure 7).

The sodic soil treatment was established using a field soil amended with high sodium, boron, aluminium and alkaline pH to represent the sodic soils in South Australia. The control soil treatment was the same soil, only unamended. Inductively coupled plasma mass spectrometry (ICP) analysis was completed on the soil treatments to confirm these were established correctly (Figure 7).

Variation in the above ground shoot phenotypes of the safflower lines was observed and the sodic treatment visibly reduced the growth of all crop types compared to the control (Figure 8). By 38 days after planting, the canola had symptoms of water stress due to becoming root bound by the 1 kg pot size. This is also apparent in the decline in the canola shoot growth seen in Figure 9. Nevertheless, the experiment was maintained to 38 days after planting to allow differentiation between the safflower lines under control and sodic treatments.

Soil Property	Soil Treatment	
	Control	Sodic
pH (1:5, soil:water)	9.30	**10.30**
pH ($CaCl_2$)	8.54	**9.02**
Calcium (%)	79.38	66.75
Magnesium (%)	12.65	8.55
Potassium (%)	2.23	1.85
Sodium (%)	5.75	**22.85**
Chloride (mg/kg)	122.50	96.00
$EC_{1:5}$ (soil:water) (dS/m)	0.19	**0.54**
Boron (mg/kg)	1.28	**12.25**
Aluminium (cmol/kg)	<0.02	<0.02

Figure 7 - The genetically modified SHO safflower, canola and wheat in a control and sodic soil in The Plant Accelerator at 38 days after planting. The ICP analysis of the soil treatments (control, sodic) confirms the high sodium, boron and alkaline pH was correctly established in the sodic treatment. Note: current aluminium soil tests focus on aluminium at acid pH and do not measure the correct speciation of aluminium (aluminate ion) at this high pH – sodium aluminate was added to the sodic treatment soil at 1.8 mg Al/kg.

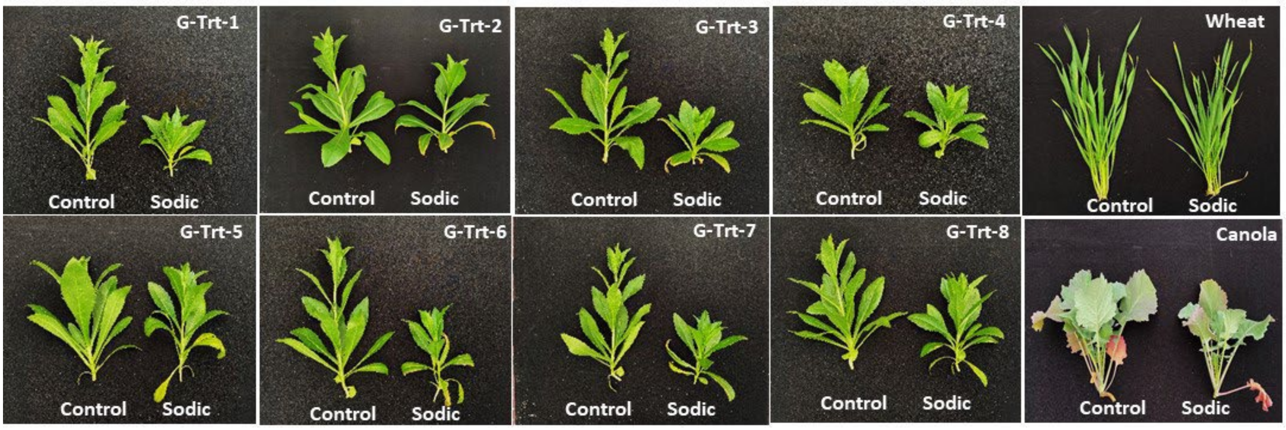


Figure 8 - A representative image of the safflower lines (G-Trt-1, G-Trt-2, G-Trt-3, G-Trt-4, G-Trt-5, G-Trt-6, G-Trt-7, G-Trt-8), wheat (cv. Mace) and canola (cv. Binito) under the control and sodic treatments at 38 days after planting.

The growth curves of the safflower, wheat and canola under the control and sodic treatment (Figure 9) indicate that the sodic treatments reduced growth of all crop types relative to control. Data has also been analysed to compare the growth of each line at the different intervals within the growth curve from day 15, 19, 23, 30, 35 and 38 (Figure 10). This shows that variation among the safflower lines in their growth under both control and sodic conditions is apparent from 35 days after planting (Figure 10). It also shows that wheat has the largest shoot biomass in the sodic treatment compared to the eight safflower lines and canola. The water use of each plant is also available, but this data is still undergoing statistical analysis at The Plant Accelerator.

The lines have been ranked according to their sodicity tolerance with variation ranging from 41 to 60% in the safflower lines to 62% for canola and 67% for wheat (Figure 11). In all crop types, shoot sodium and boron concentrations significantly increased and shoot calcium and magnesium concentrations significantly decreased in the sodic soil compared to control (Figure 12). Two safflower lines (G-Trt-2 and G-Trt-3) had significantly higher shoot boron concentrations than all other lines with values up to 236 and 248 mg B/kg respectively compared to 66 mg B/kg on average for all other safflower lines (Figure 12). These two lines with high boron concentrations were the least tolerant to the sodic soil.

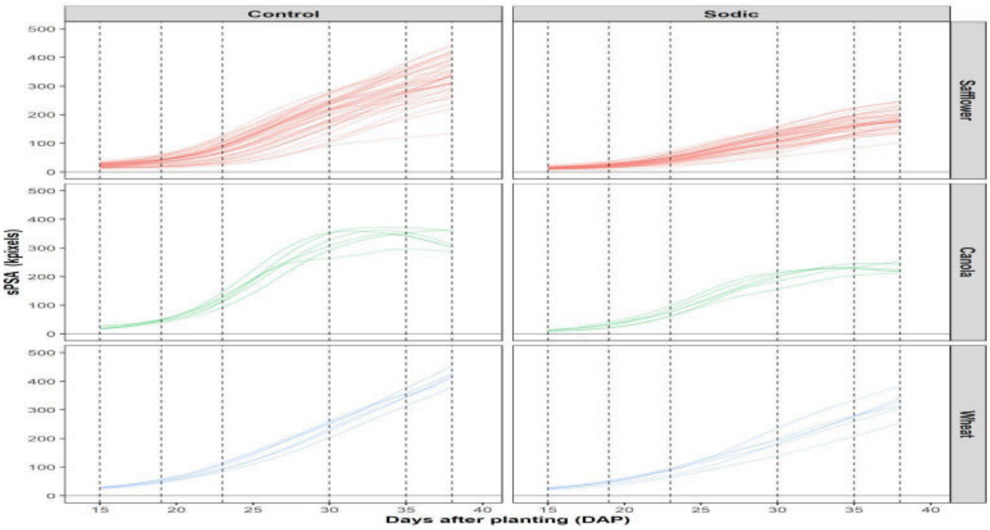


Figure 9 - Descriptive plots for growth of safflower (red), canola (green) and wheat (blue) plants at Projected Shoot Area (sPSA) with each curve an individual plant under control or sodic treatment. Vertical dashed lines correspond to days after planting (DAP) timepoints as these intervals were used for analysis.

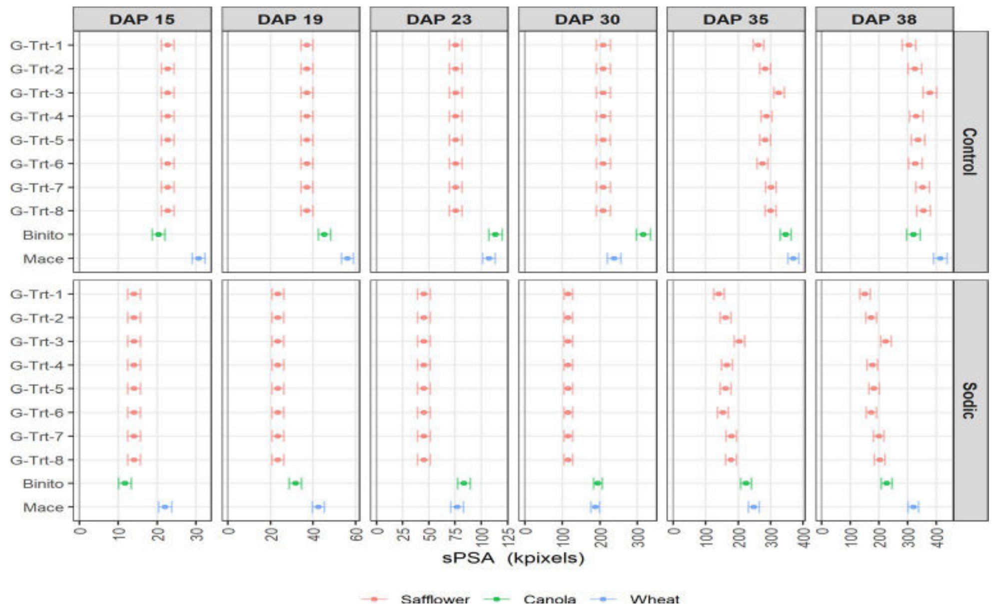


Figure 10 - Estimated marginal means over the imaging period for sPSA from 15, 19, 23, 30, 35 and 38 days after planting (DAP). Error bars correspond to half of the least significant difference [half-LSD (5%)]. If two error bars at the same DAP value and Sodicity level do not overlap, then the corresponding pair of Genotype estimated marginal means are significantly different ($pp \leq 0.05$).

Figure 11 - The mean shoot biomass of the safflower (G-Trt-1 to G-Trt-8), canola (cv. Binito) and wheat (cv. Mace) in the control (white b ar) and sodic (grey bar) treatment ordered by the sodicity tolerance of the shoot biomass under sodic relative to control (blue line) at Day 38. A different letter indicates a significant difference at P<0.001.

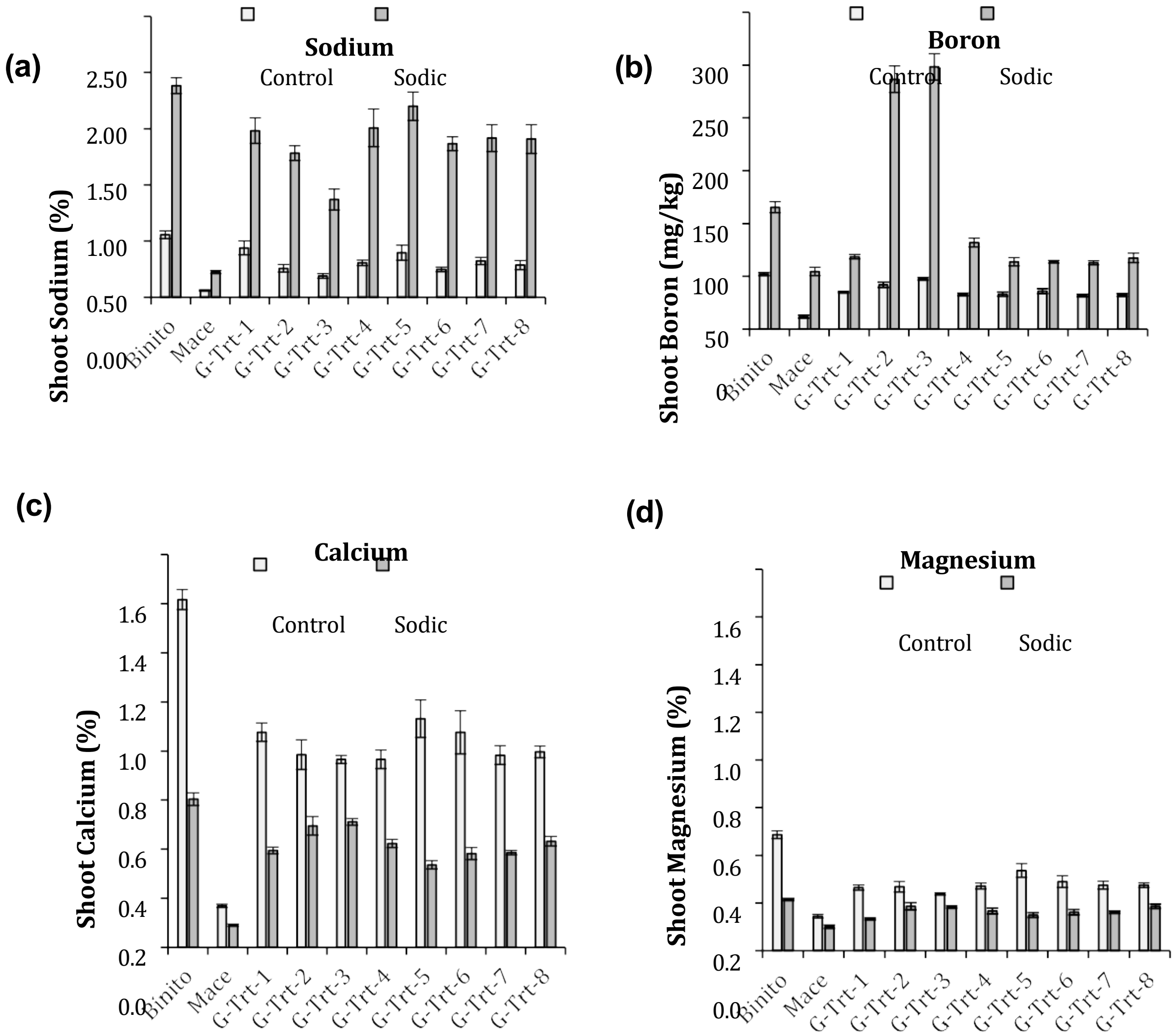


Figure 12 - The shoot tissue concentrations of (a) sodium, (b) boron, (c) calcium and (d) magnesium in the safflower (G-Trt-1 to G-Trt-8), canola (cv. Binito) and wheat (cv. Mace) under the control (white bar) and sodic (grey bar) treatments on Day 38.

CONCLUSIONS REACHED &/OR DISCOVERIES MADE

This SAGIT funded project evaluated the role of SHO safflower in saline soils and conducted a pilot experiment to determine the level of tolerance of SHO safflower lines to sodic soils (multiple constraints).

Findings indicate that SHO safflower germinated and generated shoot biomass and grain yield in both low and high salinity soil at Coomandook in a high rainfall year (2022). However, caution is needed when selecting this crop for a saline soil as failed safflower occurred due to inadequate soil moisture at seeding in a saline soil at Coomandook in 2021. There was variation amongst advanced GO Resources SHO safflower breeding lines in the high salinity site with some lines showing promise for early vigour and biomass production that warrant further investigation for use in saline soils.

A controlled greenhouse experiment determined the variation in tolerance of different SHO safflower lines to sodicity (with high sodium, high boron, high aluminium and high pH) with this ranging from 41-60 %, although this still remained less than canola (62%) and wheat (67%). Two safflower lines (G-Trt-2 and G-Trt-3) had significantly higher shoot boron concentrations than all other lines. These two lines with high boron concentrations were the least tolerant to the sodic soil. This suggests that genetic variation for boron accumulation could be selected by safflower breeders.

The results of both the field trials and the greenhouse experiment indicate that the safflower is able to establish in highly saline soils when sufficient moisture is available and that there is variation amongst SHO lines in boron concentrations and early vigour for breeders to further enhance either the sodicity and/or salinity tolerance.

INTELLECTUAL PROPERTY

There is no intellectual property generated from this project suitable for commercialisation. The SHO safflower lines are owned by GO Resources and E40-R is already commercialised for use by SA growers.

APPLICATION / COMMUNICATION OF RESULTS

This project involved field trials testing SHO safflower lines in a low and high salinity area of the same paddock at Coomandook in 2021 (dry) and 2022 (wet) year as well as a high-throughput controlled greenhouse experiment testing 8× SHO safflower lines, 1× canola (cv. Binito) and 1× wheat (cv. Mace) in a non-sodic (control) and alkaline, sodic soil with 6 replicate pots per treatment (120 pots).

Main findings

Aim 1

- The SHO safflower germinated, established biomass and grain yield in both the low and high salinity (2022 results) but care is needed as a failed safflower crop can occur if the saline soil has inadequate soil moisture (2021 results).
- The current E40-R commercially available line of the SHO safflower had variable shoot biomass in the high salinity site. There were large visual biomass differences amongst the SHO safflower lines with the advanced breeding lines being the most promising for saline soils with early vigour.
- Shoot tissue concentrations varied between low and high salinity areas with sodium concentrations increasing in safflower plants in the high salinity compared to the low salinity.
- A higher grain yield occurred in the high salinity compared to the low salinity and this is likely due to the safflower accessing legacy nutrients from previous fertiliser from seeding cereal crops that have failed to grow in this high salinity site. There was no difference in oil content between the low and high salinity safflower.

Aim 2

- Variation in tolerance to sodicity (shoot biomass in sodic relative to control soil) in the safflowers ranged from 41-60% and was less than both canola (62%) and wheat (67%).

- In all crop types, shoot sodium and boron concentrations significantly increased and shoot calcium and magnesium concentrations significantly decreased in the sodic soil compared to non-sodic control.
- Two safflower lines (G-Trt-2 and G-Trt-3) had significantly higher shoot boron concentrations than all other lines. These two lines with high boron concentrations were the least tolerant to the sodic soil. This suggests that genetic variation for boron accumulation could be selected by safflower breeders.

Publications and extension activities delivered:

- R Schilling provided an in person update at the 'Salinity Management Update and Where to fro here?' event by the Coorong Tatiara Local Action Planning Group (CTLAP) on the 22nd of March 2023 at Coomandook with 22 farmers, agronomists and advisers attending.
- R. Schilling assisted with a Stock Journal newspaper article by Quinton McCallum in 2023 describing the value of SHO safflower to South Australia. See this link: Rotational fit of SHO safflower under research lens | Stock Journal | SA
- R Schilling contributed to an article on the Plant Accelerator experiment by the Australian Plant Phenomics Facility as part of their blog updates in 2021: Evaluating SHO safflower in sodic and saline soils (plantphenomics.org.au)
- R. Schilling provided an update on twitter of the high salinity site in October 2022 with 2,716 impressions, 270 engagements and 128 detail expands.
- Direct messages from agronomists and growers in the district interested in the safflower trial was received.
- GO Resources David Hudson visited the trial site on the 20th of December 2022.

Suggested path to market

GO Resources have the commercial license for SHO safflower and have been directly linked to this project to ensure all outputs can be used to ensure a focus on salinity and sodicity tolerance within the safflower breeding program. The findings of this project indicate that the advanced SHO breeding lines look highly promising for saline soils. SA grower and adviser engagement indicates a strong interest in future use of SHO safflower and further work is needed to evaluate the advanced breeding SHO safflower lines across multiple growing seasons and multiple saline sites (from saline sands to saline clays).

POSSIBLE FUTURE WORK

The findings suggest that the SHO safflower can establish biomass and yield in a highly saline area in a high rainfall season. Given the SHO safflower was able to grow and yield in this highly saline area with high rainfall, further investigations across multiple saline sites and growing seasons to fully understand limitations and advantages of this crop type would be worthwhile to undertake. In addition, optimising the agronomic management (fertiliser, weed management, time of sowing, sowing depth and seeding rate) for the SHO safflower in a saline and sodic soil is needed for this crop type.

The ICP data from both the greenhouse (sodic) and field trial (saline) suggests there may be an opportunity to evaluate the role of different fertiliser mixes (including varied additions of calcium) on safflower grown in the saline sites. It is well established that sodium competes with calcium for uptake at the cell membrane and the nutrient testing here suggests that safflower is unable to maintain calcium concentrations and could benefit from supplementary calcium under these conditions. It is feasible that additions of calcium would further enhance the ability of the safflower to grow in the sodic and saline soils. Further work is needed to define the critical nutrient levels for safflower plants as this information is lacking.

The advanced breeding lines also indicate that these will be more vigorous than the current commercial line under saline conditions and a focus on these new lines should be a priority for future research.

Attachment 1 - The ICP analysis significant ICP results between lines for the whole shoot at branching for sodium, chloride, calcium, potassium, boron, phosphorus, sulfur, manganese, molybdenum, and zinc at low and high salinity areas in Coomandook in 2022. No significant differences detected for all lines in aluminium or iron.

Site	Line	Boron	P <.001	Calcium	P <.001	Chloride	P < 0.036	Copper	P <.001	Magnesium	P <.001	Manganese	P <.001
Low Salt	E40-R	24.3	abcd	2.3	g	2.3	abcde	2.4	ab	0.6225	cde	20.75	cdef
	SHO181	25.0	abcde	2.2	fg	1.7	ab	2.18	a	0.59	cd	17.2	abcd
	SHO44	24.1	abcd	2.2	fg	2.288	abcde	2	a	0.665	e	18.12	abcd
	SHO577	26.2	abcdefg	2.4	g	2	abcd	2.12	a	0.656	de	18.6	abcde
	X3242-2-1	23.8	abcd	2.0	def	2.275	abcde	1.975	a	0.5525	c	20.75	cdef
	X3242-4-1	23.2	abc	2.2	fg	2.4	abcde	1.98	a	0.61	cde	19.4	bcdef
	X3290-1-2-1	24.8	abcde	2.0	def	1.875	abcd	2.15	a	0.545	c	14.45	a
	X3403-6-1-1	22.7	a	2.1	efg	1.633	a	2.067	a	0.5667	cd	14.33	a
	X3403-7-3-1-1	25.0	abcdef	1.8	cde	1.907	abcd	1.8	a	0.5333	bc	15	ab
	AGR101 F1	23.0	ab	2.3	fg	1.767	abc	2.033	a	0.65	de	16	abc
High Salt	E40-R	26.3	abcdefg	1.5	ab	3.033	e	5	e	0.3633	a	23.33	ef
	SHO181	28.3	efgh	1.4	a	2.7	de	3.733	d	0.38	a	22	def
	SHO441	31.0	h	1.7	bcd	2.267	abcde	3.7	d	0.4467	ab	23.67	f
	SHO577	34.8	i	1.7	bc	2.44	acde	4.46	e	0.44	a	23	f
	X3242-2-1	28.7	fgh	1.5	ab	2.7	de	2.867	bc	0.39	a	23	ef
	X3242-4-1	27.0	bdefgh	1.5	abc	2.967	e	3.533	cd	0.3933	a	21.67	def
	X3290-1-2-1	29.7	gh	1.3	a	2.5	abcde	3.633	d	0.3867	a	21.33	cdef

Site	Line	Molybdenum	P <.001	Phosphorus	P <.001	Potassium	P <.001	Sodium	P <.001	Sulfur	P <.001	Zinc	P 0.001
Low Salt	E40-R	0.1825	a	0.2375	def	4.415	bcd	0.0402	a	0.2275	eg	20.25	de
	SHO181	0.186	a	0.222	cde	4.522	cde	0.0282	a	0.2	cdefg	18.2	cd
	SHO44	0.1675	a	0.1925	ab	4.009	bc	0.0673	a	0.1913	cdef	17.62	cd
	SHO577	0.22	a	0.228	de	4.348	bcd	0.026	a	0.224	eg	17.8	bcd
	X3242-2-1	0.1675	a	0.165	a	4.103	bc	0.0643	a	0.15	a	15.5	abc
	X3242-4-1	0.232	a	0.166	a	3.892	b	0.0316	a	0.156	ab	15.6	abc
	X3290-1-2-1	0.1675	a	0.1825	ab	4.103	bc	0.0307	a	0.1575	ab	14.75	ab
	X3403-6-1-1	0.16	a	0.1867	ab	4.103	bc	0.0223	a	0.1833	abcd	17	abcd
	X3403-7-3-1-1	0.1633	a	0.18	ab	4.147	bc	0.0423	a	0.1433	a	14.33	a
	AGR101 F1	0.1733	a	0.2033	bcd	3.197	a	0.0287	a	0.1767	abc	17.33	abcd
High Salt	E40-R	0.6133	bc	0.2767	g	5.93	g	0.5167	bc	0.2233	defg	23	e
	SHO181	0.5233	b	0.2667	fg	5.227	fg	0.117	a	0.2033	cdefg	20.67	de
	SHO441	0.6067	bc	0.2467	efg	4.997	def	0.4233	b	0.2333	g	18.33	cd
	SHO577	0.674	c	0.244	efg	5.126	f	0.62	c	0.226	eg	19	d
	X3242-2-1	0.5433	b	0.1933	abc	5.437	fg	0.4633	bc	0.1767	abc	17	abcd
	X3242-4-1	0.58	bc	0.2033	bcd	5.67	fg	0.3833	b	0.19	bcde	17	abcd
	X3290-1-2-1	0.5367	b	0.21	bcde	5.177	ef	0.46	bc	0.1933	bcdefg	17	abcd