FINAL REPORT 2015

PROJECT CODE : S12/07

PROJECT TITLE
Enhancing the grain yield and quality of oat under water deficits

PROJECT DURATION

Project Start date	1 July 2012
Project End date	30 June 2015

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Office Use Only

Project Code	
Project Type	

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PROJECT REPORT

Executive Summary

The major oat producing countries still remain in the northern hemisphere where water limiting environments are not a production constraint. Hence there is virtually no research in oat for drought tolerance globally. Australia is now the six largest oat producing country globally and with increasing domestic and export demand production will increase.

Increasing oat variety productivity in low and high rainfall regions will increase confidence to dedicated oat growers and encourage new growers to include oat as a rotation. This research project set the foundation for future research to increase variety performance in low rainfall regions, but also take advantage of increased yield in the 'good years'.

- Current milling varieties, particularly Bannister and Mitika have stable grain yield in low rainfall, but have the highest responsiveness to produce high grain yield in high rainfall.
- Chlorophyll content in leaves at four growth stages: booting, panicle emergence, anthesis, and milk development, were significantly correlated with grain yield in low rainfall and high rainfall environments. This will be incorporated as a selection tool in the National Oat Breeding Program.
- Research results will be used to improve efficiency in identifying oat varieties with higher grain yield potential in low rainfall regions, but responsive to higher grain yield for favourable growing seasons.

Project Objectives

- Identify traits that will improve the adaptation of oat to water limiting environments in South Australia and Australia allowing for more reliable production in dry seasons and regions.
- Develop and test practical phenotyping techniques.
- Introduce genetic variation using wild oat accessions in the national oat breeding program's germplasm collection.
- Identify parents to create new mapping populations for in-depth genetic studies on adaptation to water deficit.

Overall Performance

The project objectives were successfully achieved considering the complexity of the research topic. The trial was conducted with 32 entries in nine different environments that represented extreme growing conditions.

Personnel involved

Dr Pamela Zwer, Principal Investigator, Principal Plant Breeder, SARDI
Dr Victor Sadras, Crop Physiology Scientist, Climate Applications, SARDI
Dr Mahalakshmi Mahadevan, Research Officer, SARDI
Other staff members of the National Oat Breeding Program involved in the successful conduct and execution of the research trials were Sue Hoppo, Mark Hill, Kerry Lee, Peter Wheeler and Michelle Williams.

Difficulties encountered

Soil sampling: Success of soil sampling, which is important to estimate water use efficiency, depended on soil type and depth of the selected site. Soil sampling was difficult in Riverton in the 2013 season due to the hard pan and rocks as shallow as 20cm. The soil rig hit hard rocks and highly calcareous soil. Hence, water use efficiency for Riverton 2013 could not be estimated.
Thermal images: Canopy temperature estimation from thermal images needed to be captured on bright sunny days around midday during specific crop phenology. Achieving this was challenging and difficult because of weather conditions. Hence, thermal images were not captured systematically during 2013 season.

Key Performance Indicators (KPI)

KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
Review literature on drought adaptation in oat	Y	
Summarise data collected for drought adaptation, agronomic and quality traits	Y	
Interspecific hybrids produced	Y	

Summarise data collected for drought adaptation, agronomic and quality traits	Y	
Evaluate and select F2 plants from interspecific crosses	Y	
Summarise data collected for drought adaptation, agronomic and quality traits	Y	
Single seed descent for interspecific selections	N	Decided to sow as head hills in 2016
Write and submit Final Report	Y	

Technical Information

The experiments were sown at Pinery, Turretfield Research Centre (TRC), and Waikerie in 2012 and Pinery, TRC, and Riverton in 2013 and 2014. The experiments had 32 varieties and breeding lines with three replications. Data were collected for soil sampling, agronomic traits, grain yield and yield components, grain quality, hay yield and quality, and grain and biomass water use efficiency. See Appendix for Table 2 with detailed information on trials and trait assessment.

The traits were analysed to estimate the components of phenotypic variance for environment (E), genotype (G), and genotype by environment interaction (GxE). Most traits were significant for the three sources of variation, E, G, and GxE. The effect allowed us to study and understand the environmental responsiveness of the entries for all of the traits in different environments. Genotypic differences in responsiveness to grain yield in low rainfall (LR) and high rainfall (HR) environments are shown in Figure 1. Residual analysis for grain yield indicated milling varieties produce above average yields (red dots) and hay and grazing varieties produced below average yields (green and blue dots). The late hay variety, Forester, was the least responsive variety, 0.26, for grain yield, producing 0.16 t/ha in LR increasing to 2.25 t/ha in high rainfall (HR). Tungoo, a hay variety, had the highest responsiveness at 1.39. This was due to low grain yield 0.10t/ha in LR and an unusually high grain yield 5.25 t/ha in HR.

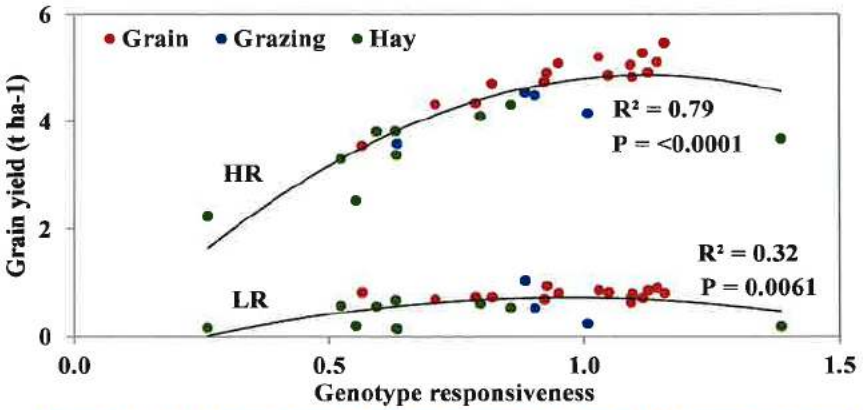

Figure 1. Yield response of genotypes to LR and HR environments

However, Tungoo yielded significantly below the average as indicated by the green dot on the far right of the figure. The milling variety, Bannister, had the second highest responsiveness of 1.16 producing 0.80 t/ha in LR, but increasing to 5.47 t/ha in HR. The milling variety, Mitika, had the next highest response of 1.12 producing 0.71 t/ha in LR increasing to 5.29 t/ha in HR. The high responsiveness indicates the varieties produced

slightly higher than the average grain yield in LR, but could take advantage of HR environments and produce higher than average grain yields. Details of analysis of other traits are presented in the Appendix.

The environment mean yield ranged from 0.3 t/ha at Waikerie (LR) to 4.4 t/ha at Riverton (HR). The top yielding lines Bannister, Mitika, Dunnart, and 05302-19 averaged approximately 3.3 t ha-1 across the environments and the lowest yielding lines were Forester 0.93t/ha, Riel 1.16 t/ha, Tammar 1.95 t/ha and Tungoo 2.06 t/ha Figure 2.

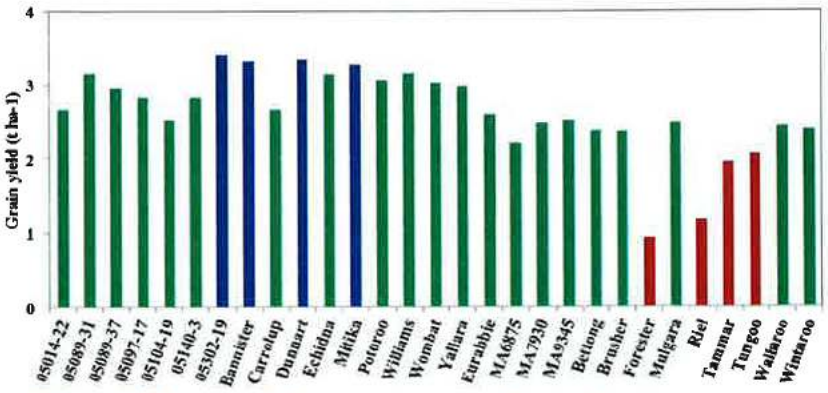

Figure 2. Mean yield of genotypes across the environments

Significant differences were observed in growing degree days (Cd) from sowing to stem elongation (SE), flowering (FL), and hay cut (HC) between the varieties/lines (G) and for SE & FL between the environments, but not for HC or GxE. Grain yield in both favourable (HR) and stressful (LR) conditions correlated with growing degree days to SE, FL, and HC. The relationship showed that the varieties/lines which attained SE before 894°Cd, FL before 1683°Cd and HC earlier than 1861°Cd produced above average yields under favourable environments. Similar thresholds were found for the stressful conditions. (See appendix for graphs).

Grain number m^{-2} and number of grains per head significantly contributed towards yield, while grain size and number of heads were not significant in the yield component analysis.

Evapotranspiration (ET) was significantly influenced by E, but not by G. Figure 3A shows the grain water use efficiency (GWUE) and Figure 3B shows the biomass water use efficiency (BWUE) of the varieties/lines and ET at four site/years. There is little variation for ET by variety within an environment, but the environments cluster separately, indicating the significance of the environment.

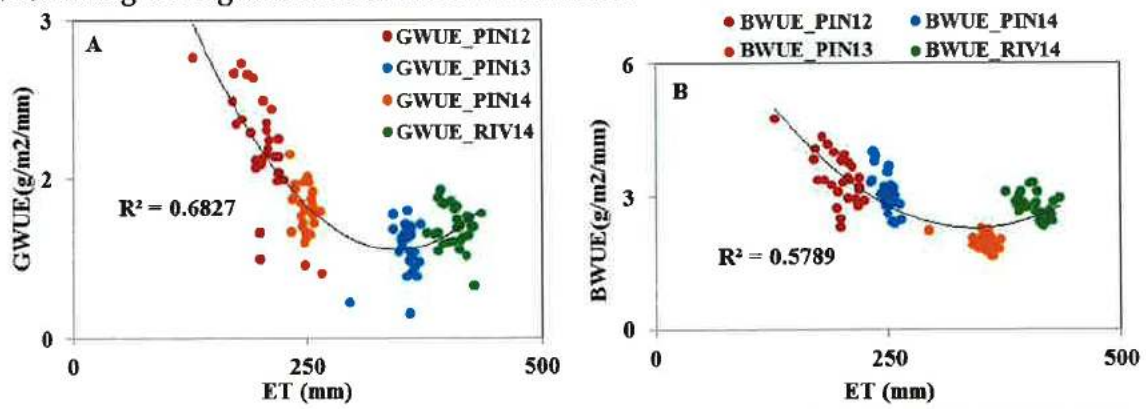

Figure 3. ET response of genotypes to environment vs GWUE (A) & BWUE (B)

GWUE and BWUE were significantly affected by E, G, and GxE and were strongly correlated with grain yield in high yield potential environments. WUE was influenced more by the genotype's biomass/grain yield than ET.

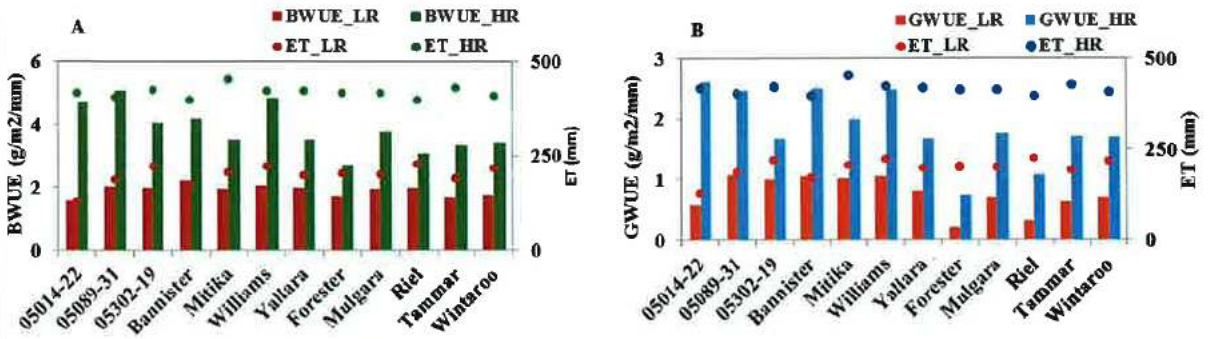

Figure 4 BWUE (A) & GWUE (B) of genotypes at HR & LR locations

BWUE and GWUE were not significant in LR. However, in HR there were significant differences between varieties/lines. The highest BWUE was observed in 05014-22 and Williams Figure 4A. The highest GWUE was 05014-22 followed by Bannister, Williams, and 05089-31Figure 4B

New phenotyping techniques were used in this research project to determine if additional trait assessment would provide more information to select breeding lines with improved performance in water limited environments. See Appendix for detailed information about SPAD, canopy temperature, and greenseeker methods. Canopy temperature had no significant correlations with grain yield or water use efficiency. The greenseeker predicted biomass accumulation up to stem elongation, but was not effective between stem elongation and flowering/grain development stages. We have used the greenseeker in the breeding program in 2015 to predict biomass or early vigour and compare to visual early vigour notes.

SPAD data were collected at four different growth stages, booting, panicle emergence, anthesis, and milk development. SPAD reflects the chlorophyll content in the leaves and can identify genotypes with the stay green character. The four growth stages were significantly correlated with grain yield and correlations were highly significant at anthesis and milk development (Figure 5). Further research will define the optimum plant stage to measure the trait in the National Oat Breeding Program.

There were 26 crosses with wild oat germplasm developed to increase genetic variation for drought tolerance. Eleven crosses were selected to promote in the program based on visual appearance. The crosses were grown in the glasshouse for two generations. Panicles were selected in the F_3 and they will be sown in headhills in 2016. See appendix for a list of the crosses and parentages.

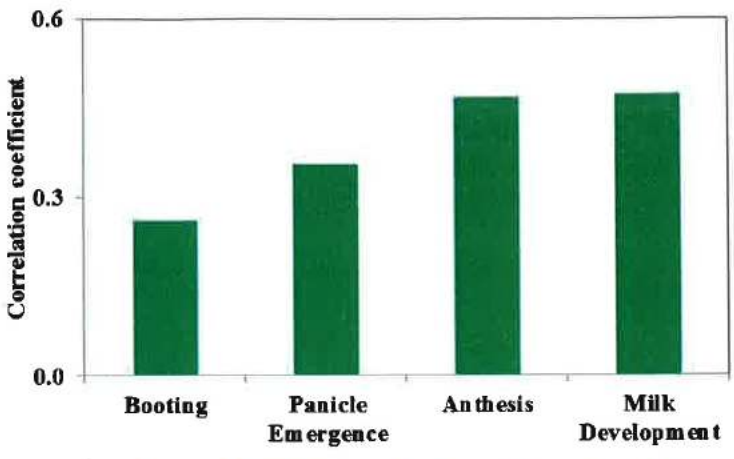

Figure 5. SPAD correlation with grain yield

Bannister had the highest responsiveness from low to high rainfall environments for grain yield. It also had the second highest GWUE of the varieties/lines assessed in the research project. The data from this study suggests that Bannister would be a good candidate for developing mapping populations for genetic studies on adaptation to water deficit.

Conclusions Reached &/or Discoveries Made

Current milling varieties are slightly higher than the average grain yield in LR, but have the responsiveness to produce high yield potential with improving environments. Growers want oat varieties that are stable in LR environments, but can be yield responsive when a good year occurs. Bannister and Mitika are two current milling varieties that combine responsiveness and high grain yield. Hay and grazing varieties are less responsive than milling varieties for grain yield. This is a likely result as the hay varieties have been selected for hay traits rather than grain yield.

Growing degree days are correlated with grain yield in LR and HR environments. The varieties selected for this study had the highest grain yield when earlier the 894°Cd for stem elongation, 1683°Cd for flowering, and 1861°Cd for hay cuts.

The yield components correlated to higher yield were grain number-m2 and number of grains per head.

Evapotranspiration differs between Pinery in 2012, 2013, and 2014 and Riverton in 2014, but varieties did not differ within a site. Varietal differences were found for GWUE and BWUE in HR, but not LR.

New phenotyping techniques to improve selection efficiency for improved performance in water limited environments were assessed. Canopy temperature measured by infrared photographs was not correlated with improved grain yield. Greenseeker was useful until growth stage 31 to evaluate early vigour. Leaf chlorophyll content measured by SPAD was correlated with increased grain yield in LR and HR environments at all four growth stages assessed.

Two current milling varieties were identified as potential parents for developing mapping populations to further research the genetic basis of drought tolerance.

Intellectual Property

The milling varieties used in the study were released and commercialized prior to this research project. The information generated will be utilized in the National Oat Breeding Program to improve future oat variety releases for drought tolerance.

Application / Communication of Results

Main Findings:

- Current milling varieties, particularly Bannister and Mitika have stable grain yield in LR, but have the highest responsiveness to produce high grain yield in HR.
- Chlorophyll content in leaves at four growth stages; booting, panicle emergence, anthesis, and milk development were significantly correlated with grain yield in LR and HR environments. This will be incorporated into data collection in the National Oat Breeding Program.
- Research results will be used to improve efficiency in identifying oat varieties with higher yield potential in LR, but responsive to higher grain yield in favourable growing seasons.

Improved adaptation to drought along with responsiveness to favourable growing years will provide growers with both stable and responsive oat varieties. Dry conditions experienced at the various growth stages especially at the end of the growing season across southern Australia and the movement of oat production into traditional low rainfall regions require oat varieties with stability and responsiveness. Increasing grower confidence in oat varieties that perform in LR and HR environments will increase the area sown to oats for a consistent and stable supply for the milling industry.

Results were reported at the Elmore and Hart Field Days, and Grains Industry Western Australia Oat Field Days. Progress was reported in the Oat Newsletter in 2013, 2014, and 2015 available on the PIRSA website under SARDI, Sustainable Systems. Dr. Mahalakshmi Mahadevan prepared a paper and will present the research at the Agronomy Conference in Hobart in 2015. Numerous peer reviewed papers will be prepared and submitted. The accepted papers will be forwarded to SAGIT.

POSSIBLE FUTURE WORK

The new phenotyping technique using SPAD will require fine tuning, so that the optimum growth stage is determined for assessment of F_5 to F_8 lines in the National Oat Breeding Program. The wild oat crosses will be assessed in the field in 2016 to determine if the grain yield potential can be increased above current milling varieties in LR environments. Mapping populations will be developed so when funding is available, research for makers linked to improved drought tolerance can be implemented. It takes approximately four to five years to develop an oat mapping population.

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S12/07: Enhancing the grain yield and quality of oat under water deficits

Final report to SAGIT

August 2015

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Dr Mahalakshmi Mahadevan

Dr Victor Sadras

South Australian Research and Development Institute

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Summary

Introduction

The SAGIT approved project on ENHANCING THE GRAIN YIELD AND QUALITY OF OAT UNDER WATER DEFICITS started in July 2012 as a three years project with the following objectives

- Identify traits that will improve the adaptation of oat to water limiting environments in South Australia and Australia allowing for more reliable production in dry seasons and regions.
- Develop and test practical phenotyping techniques.
- Introduce genetic variation using wild oat accessions in the National Oat Breeding Program's germplasm collection.
- Identify parents to create new mapping populations for in-depth genetic studies on adaptation to water deficit.

Project Staff

The project was instigated by Dr. Pamela Zwer, Principal Plant Breeder, SARDI as principal investigator based at the Waite. Dr. Victor Sadras, Climate Applications, SARDI, was involved in the project for the drought tolerance aspects of the project. Dr. Mahalakshmi Mahadevan was appointed as Research Officer for the conduct of the trial.

Other staff members of the National Oat Breeding Program involved in the successful conduct and execution of the research trials were Sue Hoppo, Mark Hill, Kerry Lee, Peter Wheeler and Michelle Williams

Materials and Methods

Test environments and experimental layout

Twenty nine oat entries were chosen for evaluation over three crop seasons (2012, 2013 & 2014) in three different locations each season in Lower North and Mid North regions of South Australia. The entries evaluated consisted of advanced breeding lines, released varieties of grain, hay and grazing types which varied in height, growth habit and maturity **Table 1**. Crops were sown in randomised complete block design with three replications.

A trial environment was a combination of year and location. The environments differed widely for the meteorological conditions and were considered to be high, medium and low rain fall regions based on mean annual rainfall. Details of the environments are provided in **Table 2**. The environments differed widely for the meteorological conditions and were considered to be high, medium and low rain fall regions based on mean annual rainfall.

Plot size was 4.16m^2 (3.2 m x 1.3m) for Pinery, Turretfield and Riverton sites during all the years. Sowing was taken at a seed rate of 165 seeds / m2 with five rows of crop at 0.22m spacing in each plot. Crops were fertilized with 120kg/ha of diammonium phosphate. Plots size at Waikerie was 7.2 m^2 (5 m x 1.44 m), with six rows spaced at 0.254m. Seed rate was 180 seeds /m^2. All other agronomic practices, seed treatment, fertilizer, pest management and herbicide application were carried out in accordance to the specific requirements of each environment, except for disease management.

Four entries, Euro, Glider, Kangaroo and Mortlock (highlighted in **Table** 1)were not uniform for all the trial sites, hence were disregarded for statistical analysis to keep the genotypes uniform between the environments. Details of different traits observed are detailed in (A & B)

Observations

Soil sampling

Initial soil samples at sowing were collected using a mechanically driven soil rig at 20cm intervals up to one meter depth. Soil moisture was estimated by gravimetric method and expressed as volumetric water content (mm). Soils were analysed for soil characteristics (by CSBP, WA); EC, pH, Boron, sodium, nitrate nitrogen, ammonical nitrogen, bulk density, soil type and classification. Final soil samples were collected by adopting the same procedure for each plot representing one genotype, to a depth of 1m to estimate volumetric soil moisture content (mm). Evapotranspiration (ET) of individual genotypes was estimated from the initial and final soil moisture content and the amount of rainfall received from sowing to harvest. ET was used to estimate Water Use Efficiencies (WUE); biomass water use efficiency (BWUE) and grain water use efficiency (GWUE)

Phenology: The crop growth stages were scored at weekly intervals using Zadoks scales to estimate days taken to and thermal degree days to the critical stages; stem elongation (SE), flowering (FL) and hay cutting stage (HC)

Establishment: plant population was counted around six weeks after sowing and were expressed as numbers per m^2

Tiller count: Tiler counts were at SE (Zadoks 31) and HC (Zadoks 71) and expressed as number per m^2

Greenseeker: Greenseeker (Greenseeker Hand Held optical Sensor Unit, model 505, NTech Industries, Inc. California, USA) was used to measure the NDVI (Normalised Difference Vegetative Index) of individual lines at fortnightly intervals from eight weeks after sowing at fortnightly intervals through to harvest during 2014 season and until a point when NDVI values started to decline during 2014 season. These observations where used to project the biomass of the genotype from the regression equation obtained from the calibration of NDVI and biomass of selected lines.

Canopy temperature: Thermal images were captured using FLIR B365 camera with 25 °C during clear still day days, preferably between 11 am to 2 pm and further processed using FLIR quick reporter software. Four crop growth stages were selected to capture the images: half panicle emergence (Zadoks 55), panicle emergence completed (Zadoks 59), flowering (Zadoks 60 – 65) and grain development (Zadoks 70-77). There were practical difficulties in capturing images at the right stage of crop growth during 2013 season. However, they were successfully captured during 2014 season. Five images were captured in Pinery and six in Riverton while only three and four images were used for the sites respectively since all varieties were captured on the same day.

SPAD: Handheld Chlorophyll meter SPAD502 Plus, measures the relative amount of chlorophyll present in the leaves, which served as an overall indicator of plant health and "stay green status". SPAD values of individual plots were recorded in flag leaf at four important growth stages, booting as SPAD 1 (Zadoks 41-50), panicle emergence as SPAD 2 (Zadoks 51-60), flowering as SPAD 3 (Zadoks 61-70) and milk development as SPAD 4 (Zadoks 71-77).

Hay yield: Biomass cuts from 0.5m length of the centre three rows (total of 1.5 m) were taken from individual plots during stage milk development stage (Zadoks 71) and hay yields were estimated and expressed as g/m2. Biomass cuts of 0.5 m lengths from two border rows (making up 1m length in total) were taken separately to the centre rows to estimate the yield parameters only. This was done for one of the locations, Riverton. The biomass was collected in individual paper bags, labelled and subsequently dried in the oven at 50-60°C in the oven for 3 days to record dry weight. Subsequently biomass per m^2 would be determined.

Plant height: Plant height was measured at physiological maturity (before harvest).

Final biomass: Quadrant samples (0.5m x 3 rows) were collected from individual plots (genotypes) to estimate total biomass.

Yield and Quality: The quadrant samples collected for biomass estimation were used to determine yield and yield components, grain physical and NIR qualities.

The traits observed &/ computed, abbreviations and the units they are expressed are listed in Table 4 Abbreviations and units of traits measured &/ computed

Table 1. List and description of oat genotypes used for the study

Entry no.	Name	Type	Height	Growth habit	Maturity	Greenseeker calibration variety
1	Bannister	Grain	Tall Dwarf	Semi-erect	Mid	W
2	Bettong		Medium Tall	Semi-erect	Mid	Y
3	Brusher		Tall	Semi-erect	Early-mid	Y
4	Carrolup		Medium Tall	Semi-erect	Early-mid	Y
5	Dunnart	Grain	Tall Dwarf	Semi-erect	Early-mid	W/M
6	Echidna	Grain	Dwarf	Semi-erect	Mid	M
7	Eurabbie	Grazing	Dwarf	Semi-erect	Mid-late	MA
8	Euro	Grain	Medium Tall	Erect	Early-mid	NOT USED
9	Forester		Tall	Semi-erect	Late	F
10	Glider	Hay	Medium Tall	Semi-erect	Late	G
11	Kangaroo	Hay	Medium Tall	Semi-erect	Mid-late	G
12	Mitika	Grain	Dwarf	Semi-erect	Early	M
13	Mortlock	Grain	Medium Tall	Semi-erect	Early-mid	Y
14	Mulgara		Tall	Semi-erect	Mid	Y
15	Potoroo	Grain	Tall Dwarf	Semi-erect	Early-mid	W/M
16	Riel		Medium Tall	Semi-erect	Late	F
17	Tammar		Medium Tall	Semi-erect	Mid-late	Y
18	Tungoo		Tall	Erect	Mid-late	Y
19	Wallaroo		Tall	Semi-erect	Early	Y
20	Williams	Grain	Medium Tall	Semi-erect	Early-mid	W
21	Wintaroo		Tall	Semi-erect	Mid	Y
22	Wombat	Grain	Dwarf	Semi-erect	Mid	M
23	Yallara	Grain	Medium Tall	Erect	Early-mid	Y
24	MA6875	Grazing	Dwarf	Prostrate	Mid-late	MA
25	MA7930	Grazing	Dwarf	Prostrate	Mid-late	MA
26	MA9345	Grazing	Dwarf	Prostrate	Mid-late	MA
27	05014-22	Grain	Tall	Semi-erect	Early	Y
28	05089-31	Grain	Tall Dwarf	Semi-erect	Mid	W/M
29	05089-37	Grain	Tall Dwarf	Semi-erect	Early	W/M
30	05097-17	Grain	Medium Tall	Erect	Early	Y
31	05104-19	Grain	Tall	Erect	Early	Y
32	05140-3	Grain	Medium Tall	Erect	Very early	Y
33	05302-19	Grain	Medium Tall	Erect	Early	Y

Lines used for greenseeker calibration
MA6875 designated as MA
Yallara designated as Y
Forester designated as F
Glider designated as G
Mitika designated as M
Williams designated as W

Table 2. Details of the trial environments (season & location) selected for evaluation of 29 oat entries

Season	Location	Environment	Rainfall zone	T_{Max} (℃)	T_{Min} (℃)	Rainfall during the growing season (mm)	Date of sowing	GPS Coordinates	Meteorological station (No.)	Evapotranspiration during the growing period (mm)
2012	Turretfield	TRC12	Medium	11.0 to 40.6	-1.0 to 25.5	236.6	8th June	34°32′ 35.38″S 138°49′ 32.46″E	Rosedale (23343)	606.1
	Pinery	PIN12	Low	12.0 to 40.0	-0.5 to 24.5	199.8	5th June	34°19′ 44.48″S 138°28′ 51.98″E	Owen (23012)	549.3
	Waikerie	WAK12	Low	12.5 to 39.5	-4.5 to 25.0	75.3	30th May	34°15′ 19.8″S 140°0′ 08″E	Waikerie (24018)	486.6
2013	Riverton	RIV13	High	11.0 to 40.0	0.5 to 20.5	365.9	29th May	34° 12′ 01.30″ S 138° 44′ 24.29″ E	Riverton (23314)	547.0
	Turretfield	TRC13	Medium	10.7 to 39.8	0.4 to 24.8	262.1	25th June	34°32′ 36.20″ S 138°49′19.53″ E	Rosedale (23343)	512.3
	Pinery	PIN13	Low	11.5 to 38.0	1.0 to 17.0	257.1	28th May	34°19′ 24.27″ S 138°29′14.00″ E	Owen (23012)	423.7
2014	Riverton	RIV14	High	11.0 to 41.0	-1.5 to 22.5	282.7	30th May	34° 13′ 11.13″ S 138° 44′ 3.08″ E	Riverton (23314)	627.6
	Turretfield	TRC14	Medium	10.5 to 41.4	-1.4 to 24.2	251.8	6th June	34°32′ 59.10″S 138°50′ 28.30″E	Rosedale (23343)	525.4
	Pinery	PIN14	Low	11.5 to 41.5	-1.0 to 22.5	174.0	26th May	34°20′ 39.90″ S 138°29′23.65″ E	Owen (23012)	526.0

Table 3 A. Trial location, season, experiment details and various traits observed

Season	Location	Environment	Experiment		Soil sampling			Agronomic traits						
			Number of entries	Number of replications	Initial	Final	EM mapping	plant population /m2	Tiller Count /m2	Tiller Count /m2 (GS71)	Phenology	Plant Height (cm)	SPAD	Canopy temp
2012	PIN	PIN12	32	3	√	√	×	×	×	×	×	√	√ (1)	×
	TRC	TRC12	30	2	×	×	×	×	×	×	×	×	√ (1)	×
	WAK	WAK12	30	3	×	×	×	×	×	×	×	√	×	×
2013	PIN	PIN13	32	3	√	√	√	√	√ (60 DAS)	√	√	×	√ (4)	×
	RIV	RIV13	32	3	√	×	√	√	√ (60 DAS)	√	√	×	√ (4)	×
	TRC	TRC13	32	3	√	×	×	×	×	×	×	×	×	×
2014	PIN	PIN14	32	3	√	√	√	√	√ (GS 31)	√	√	√	√ (5)	√ (8)
	RIV	RIV14	32	3	√	√	√	√	√ (GS 31)	√	√	√	√ (6)	√ (8)
	TRC	TRC14	32	3	√	×	×	×	×	×	×	×	×	×
No. of environments					7	4	4	4	4	4	4	4	4	2

Table 3 B. (Conti.) Trial location, season, experiment details and various traits observed

Season	Location	Environment	Hay		Final biomass		Yield & yield components						Grain Quality	
			Yield	NIR	Weight (g/m2)	NIR	Grain Yield	Grain Number	Grain Size	Number of heads	Number of grains /head	Head weight	Physical	NIR
2012	PIN	PIN12	√	√	√	√	√	√	√	√	√	√	√	√
	TRC	TRC12	×	×	×	×	√	√	√	×	×	×	√	√
	WAK	WAK12	×	×	×	×	√	×	×	×	×	×	×	×
2013	PIN	PIN13	√	√	√	√	√	√	√	√	√	√	√	√
	RIV	RIV13	√	√	√	√	√	√	√	√	√	√	√	√
	TRC	TRC13	×	×	×	×	√	×	×	×	×	×	×	√
2014	PIN	PIN14	√	√	√	√	√	√	√	√	√	√	√	√
	RIV	RIV14	√	√	√	√	√	√	√	√	√	√	√	√
	TRC	TRC14	×	×	×	×	√	√	√	×	×	×	√	√
No. of environments			5	5	5	5	5	5	7	5	5	5	7	8

Table 4 Abbreviations and units of traits measured &/ computed

Traits measured &/ computed	Abbreviations	Units
Water Use Efficiency	WUE	
Evapotranspiration	ET	Mm
Biomass water use efficiency	BWUE	
Grain water use efficiency	GWUE	
Plant population	EST	Number /m2
Tiller counts	TC	Number /m2
Plant Phenology scores		Zadoks scale
Days to SE (Zadoks 31)	Days_SE	Number
Days to FL (Zadoks 60)	Days_FL	Number
Days to HC (Zadoks 71)	Days_HC	Number
Thermal degree days to SE (Zadoks 31)	Cd_SE	Degree days
Thermal degree days to FL (Zadoks 60)	Cd_FL	Degree days
Thermal degree days to HC (Zadoks 71)	Cd_HC	Degree days
Hay yield	HY	g/m2
Crude protein	HCP	%
Water soluable carbohydrates	HWSC	%
Nitrogen	HN	%
Digestibility	Hdig	%
Metabolisable energy	HME	%
Acid detergent fiber	HADF	%
Neutral detergent fiber	HNDF	%
SPAD	SPAD	value
NDVI	NDVI	value
Canopy temperature	CT	°c
Plant height	PH	Cm
Yield & Yield components		
Grain Yield	GY	t/ha
Grain number	GN	Number
Number of heads	NOH	Number
Head weight	HW	g/m2
Number of grains per head	NOG/H	Number
Grain size(1000 grain weight)	GS	G
Hectolitre weight	HLW	Kg
screening	SCR	%
Protein	PRO	%
Oil	OIL	%
Groat	GRO	%
β-Glucan	βGlu	%
Biomass at harvest	BHAR	g/m2
Harvest Index	HI	
Dry matter	SDM	%
Straw Crude protein	SCP	%

Straw Water soluable carbohydrates	SWSC	%
Straw Nitrogen	SN	%
Straw Digestibility	Sdig	%
Straw Acid detergent fiber	SADF	%
Straw Neutral detergent fiber	SNDF	%

Results and discussion:

All the traits observed (**Table** 3 A &B) and those computed from primary observations (Table 4) were statistically analysed to estimate the traits for phenotypic variance; Environment (E), Genotype (G) and G x E interaction. All the above three sources of variation had significant influence on most of the traits with P values < 0.05. This indicated the diversity of the genotypes and the environments selected for the study. It also allowed us to study and understand the response (**plasticity**) or behaviour of the genotypes to the environment which were determined statistically through variance ratio (**VR**). The responsiveness of a trait was environment dependent. The strength of correlation of VR (trait plasticity) as indicated by the R^2 values, was the strongest in the high rainfall (HR) (90^{th} percentile) and weakest in the low rainfall (LR) (10^{th} percentile). Higher the VR, higher was the responsiveness of a genotype to the environment. Regression and residual analysis of the correlation helped us to study the strength of association between the traits and their responsiveness and if it were positively or negatively contributing towards the trait at HR & LR locations.

Grain yield (GY)

RIV13 registered the highest (365mm) and WAK12 the lowest (75mm) rainfall from sowing to harvest. The difference between the evaporative demand and rainfall was the highest for WAK12 (411mm) and lowest for RIV13 (263). Grain yield was analysed for nine environments. The environment mean yield ranged from 0.3 t/ha at Waikerie (LR) to 4.4 t/ha at Riverton (HR). The top yielding lines Bannister, Mitika, Dunnart, and 05302-19 averaged approximately 3.3 t ha-1 across the environments and the lowest yielding lines were Forester 0.93t/ha, Riel 1.16 t/ha and Tammar 1.95 t/ha.

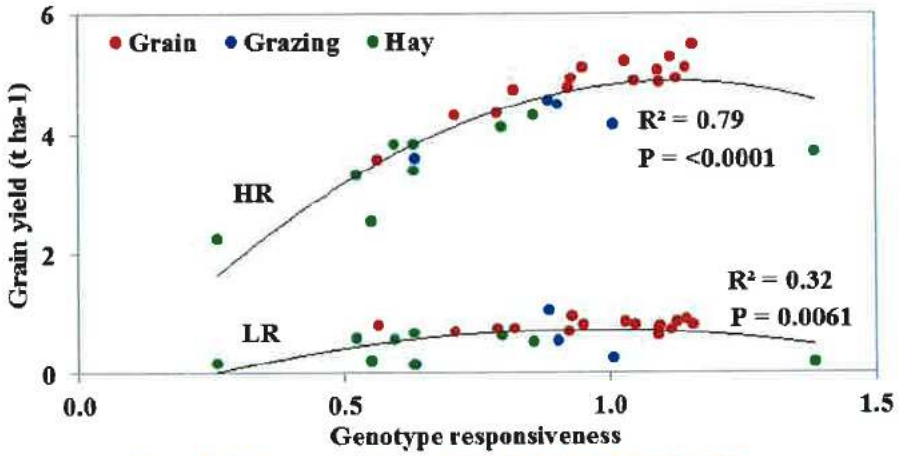


Figure 1 yield response of genotypes to HR and LR conditions

Genotypic differences in responsiveness to grain yield in LR and HR environments are shown in Figure 1. Residual analysis for grain yield indicated milling varieties produce above average yields (red dots) and hay and grazing varieties produced below average yields (green and blue dots). The late hay variety, Forester, was the least responsive variety, 0.26, for grain yield, producing 0.16 t/ha in low rainfall (LR) environment, increasing to 2.25 t/ha in high rainfall (HR). The milling variety, Bannister, had the highest responsiveness of 1.16 producing 0.80 t/ha in LR, but increasing to 5.47 t/ha in HR. The milling variety, Mitika, had the next highest response of 1.12 producing 0.71 t/ha in LR increasing to 5.29 t/ha in HR. The high responsiveness indicates the varieties produced slightly higher than the average grain yield in LR, but could take advantage of HR environments and produce higher than average grain yields.

Significant differences were observed in growing degree days (Cd) from sowing to stem elongation (SE), flowering (FL), and hay cut (HC) between the varieties/lines (G) and for SE

& FL between the environments, but not for HC or GxE (Table 5). Grain yield in both HR and LR conditions significantly correlated with growing degree days to SE, FL, and HC. The relationship showed that the varieties/lines which attained SE before 894°Cd, FL before 1683°Cd and HC earlier than 1861°Cd produced above average yields under favorable environments (Figure 2). Similar thresholds were found for the stressful conditions.

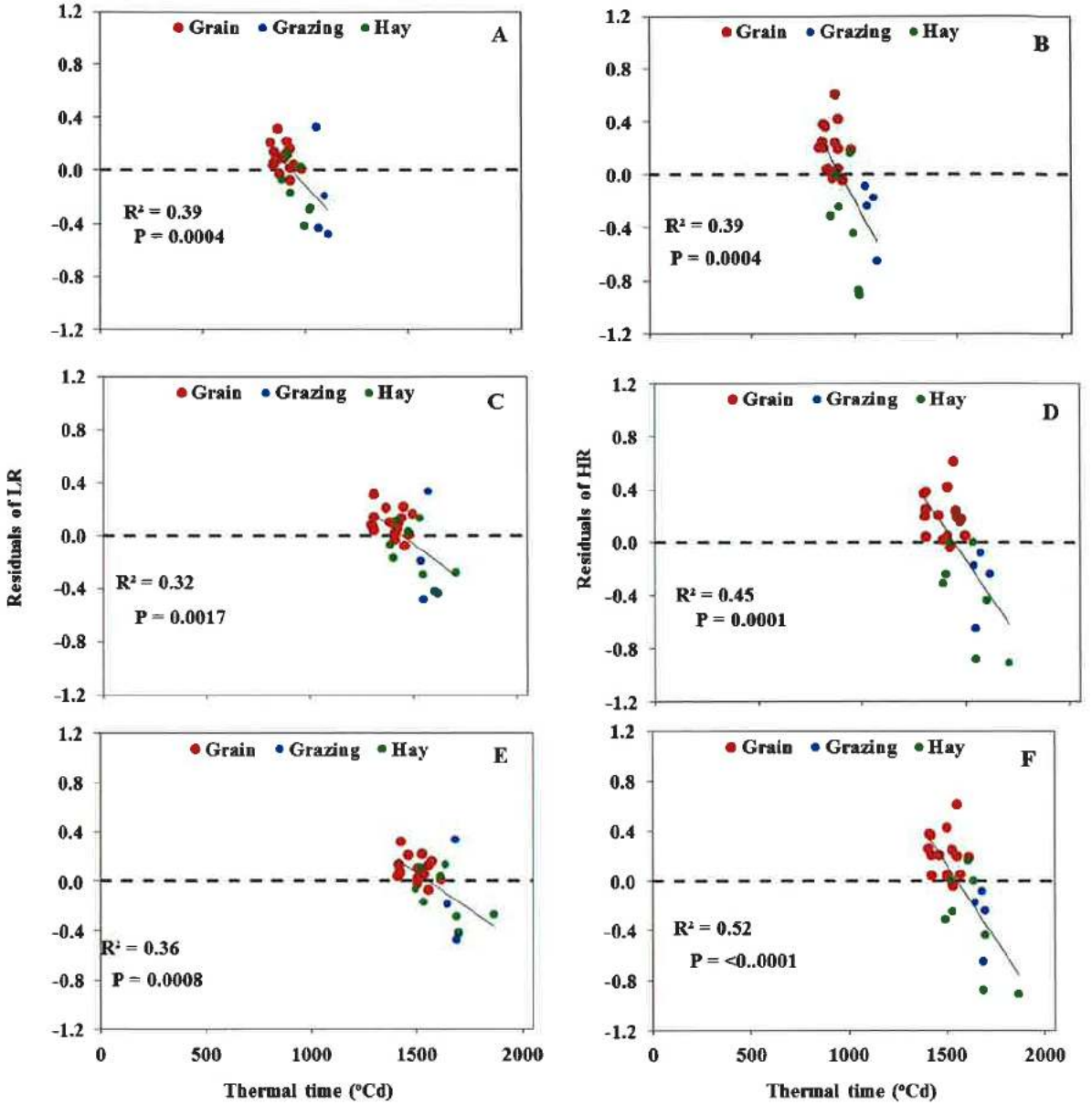


Figure 2 Relationships between residuals of yield vs responsiveness at LR and HR environments of 29 entry's growing degree days from sowing to SE (A & B), FL (C & D) and HC (E & F). Forester, an extremely late variety was excluded from the analysis

Yield components (GN, GS, NOH & NOG/H)

ANOVA for yield components; GN, GS, NOH & NOG/H (Table 5) showed highly significant influence of E, G and G x E for all the above components ($P < 0.0001$). Yield components were analysed for responsiveness (plasticity/VR) of genotypes to LR and HR locations and presented in Table 6 Responsiveness of yield components & grain physical and NIT qualities to LR and HR. GN, NOH & NOG/H had very highly significant correlation with HR locations and were non-significant (NS) at LR locations. While, GS was exactly opposite, significantly correlated at LR

and NS for HR locations. This means that owing to poor number of grain set, low number of heads and less number of grains per head (**SINK**) under LR conditions favoured better GS.

Table 5 P values of ANOVA for E, G, G x G for various traits

	GY (9 E)	GY (7E)	GN (7E)	GS (7E)	NOH (5E)	NOG/H (5E)	HLW (5E)	SCR % (5E)	Protein % (8E)	Oil % (8E)	Groat % (8E)	β-Glucan (8E)
	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value
Environment (E)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Genotype (G)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G x E	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

	HI (5E)	HY (5E)	HWSC (5E)	HN (5E)	Hdig (5E)	HME	HADF	HNDF	SWSC (5E)	SN (5E)	Sdig	SSDF	SNDF
	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value
Environment (E)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Genotype (G)	<0.0001	<0.0001	<0.0001	0.0067	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
G x E	<0.0001	0.0122	<0.0001	0.0046	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NS	0.0001	<0.0001	<0.0001

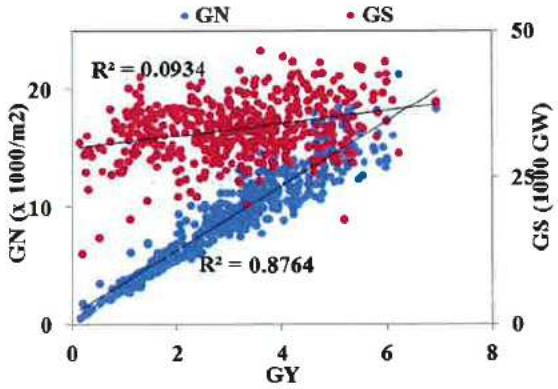
	PH (4E)	Est (4E)	Tillers: GS 71 & GS31 (2E)	Tillers: GS71 & harvest (4E)	Cd_SE (4E)	Cd_FL (4E)	Cd_HC (4E)	Cd_SE (4E)	Cd_FL (4E)	Cd_HC (4E)	Days_SE (4E)	Days_FL (4E)	Days_HC (4E)	Days_SE (4E)	Days_FL (4E)	Days_HC (4E)
	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value
Environment (E)	<0.0001	<0.0001	NS	<0.0001	<0.0001	0.0055	NS				<0.0001	<0.0001	<0.0001			
Genotype (G)	<0.0001	<0.0001	<0.0001	<0.0001				<0.0001	<0.0001	<0.0001				<0.0001	<0.0001	<0.0001
G x E	<0.0001	<0.0001	0.002	<0.0001												

	Cd SE (4E)	Days_SE (4E)	Days_FL (4E)
	P-Value	P-Value	P-Value
Environment (E)	<0.0001	<0.0001	<0.0001
Genotype (G)	<0.0001	<0.0001	<0.0001
G x E	<0.0001	<0.0001	<0.0001

	SPAD 1 (4E)	SPAD 2 (4 E)	SPAD 3 (4E)	SPAD 4 (4E)	SPAD 5 (2E)	SPAD 6 (1E)	ET (4E)	BWUE (4 E)	GWUE (4E)
	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value
Environment (E)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001
Genotype (G)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	NS	<0.0001	<0.0001
G x E	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		NS	0.0007	<0.0001

Table 6 Responsiveness of yield components & grain physical and NIT qualities to LR and HR

Traits	LR		HR	
	P values	R2	P values	R2
GN	NS	-	<0.0001	0.664
GS	0.0187	0.264	NS	-
NOH	NS	-	0.009	0.304
NOG/H	NS	-	<0.0001	0.795
HLW	0.0001	0.424	0.0214	0.181
SCR	<0.0001	0.444	<0.0001	0.906
PRO	NS	-	0.0004	0.379
OIL	NS	-	0.0007	0.351
GRO	0.0002	0.399	NS	-
βglu	<0.0001	0.627	NS	-



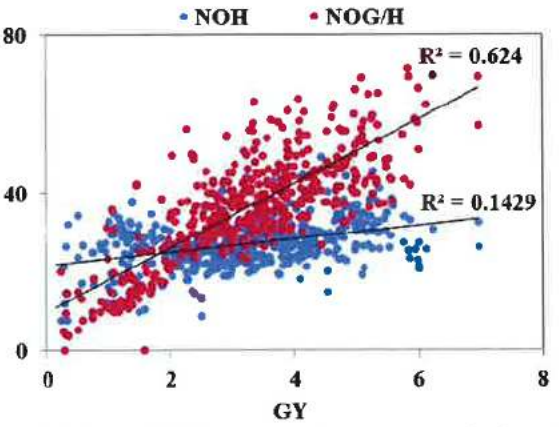


Figure 3 Association of GY with yield components: GN, GS, NOH & NOG/H

Correlation analysis between yield and yield components revealed GN and NOG/H were main contributors for GY than GS and NOH (**Error! Reference source not found.**). Correlation analysis showed zero correlation of GS with GY while GN had the strongest value of 1 (Table 7). A negative correlation relationship was evident between GS & GN and GS & NOG/H, which means higher the GN and NOG/H lower, is the GS.

Grain Quality

E, G & G x E significantly influenced the grain physical and NIR qualities; hectolitre weight (HLW), Screening (SCR), protein (PRO), oil (OIL), groat (GRO) and β glucan (βglu) (Table 5). Responsiveness (plasticity/VR) of genotypes to LR and HR conditions showed significant results for HLW & SCR under both the conditions while significant response was observed for PRO & GRO to HR and GRO & βglu to LR (Table 6). Williams noticeably had the highest SCR among the grain varieties. SCR also had significant negative correlation with GS, HLW and GRO. Similar relationship existed between PRO and HLW & OIL(Table 7).

Table 7. Correlation of yield components and grain physical and NIR qualities with GY

	GY	GN	GS	NOH	NOG/H	HLW	SCR	PRO	OIL	GRO	βGlu
GY	1										
GN	1.0	1									
GS	0.0	-0.1	1								
NOH	0.5	0.5	0.1	1							
NOG/H	0.9	0.9	-0.2	0.1	1						
HLW	0.2	0.0	0.6	0.1	0.0	1					
SCR	0.3	0.4	-0.7	0.0	0.5	-0.5	1				
PRO	-0.2	-0.2	0.0	0.3	-0.4	-0.3	0.0	1			
OIL	-0.1	-0.1	0.1	-0.1	-0.1	0.4	-0.2	-0.4	1		
GRO	0.2	0.1	0.5	0.3	0.1	0.5	-0.4	-0.1	0.2	1	
βGlu	-0.1	-0.1	0.0	0.3	-0.2	-0.1	-0.2	0.3	0.0	0.2	1

P values	0.001	0.01	0.05

Hay yield and quality; Harvest index (HI)

Hay yield was significantly influenced by E, G & G x E (Table 5). Hay yield response to the environment did not show any pattern of response whether the genotypes were grain or hay types. Though hay quality traits were significantly influenced by the E, their response to HR was more pronounced than to LR.

HI (yield divided by total biomass produced) was significantly influenced by the three sources of variation (Table 5) with the responsiveness of genotypes to HR conditions alone being significant. The residual effects were similar to that of GY with grain varieties being positive than hay or grazing types. The reason being HI was determined more by GY than by biomass as evident by the correlation coefficient values in Table 8.

Table 8 Correlation of GY with HI and hay & straw yield and quality

	GY	HI	HY	HWSC	HN	SWSC	SN
GY	1						
HI	0.9	1					
HY	0.5	0.2	1				
HWSC	0.1	0.0	0.3	1			
HN	0.3	0.0	0.8	-0.2	1		
SWSC	0.0	-0.1	0.0	0.8	-0.3	1	
SN	-0.3	-0.5	0.2	-0.2	0.5	-0.3	1

Agronomic traits

Plant height

Plant height was influenced by three sources of variation). Average plant height across environments ranged from 45 to 99 cm. Plasticity of plant height ranged from 0.43 to 1.64 and

correlated with HR but not with LR Figure 4. This means plasticity resulted from responsiveness to favourable conditions with no systematic variation among lines under stress. Average yield was non-linearly related to plant height and there was a negative relationship between plasticity of yield and plasticity of height (Figure 5A) Analysis of plant height is vital in breeding as this trait influences harvest index and yield (Figure 5B) with dwarf genotypes possessing greater yield potential than the tall genotypes. However, the tall genotypes have greater potential for hay yield

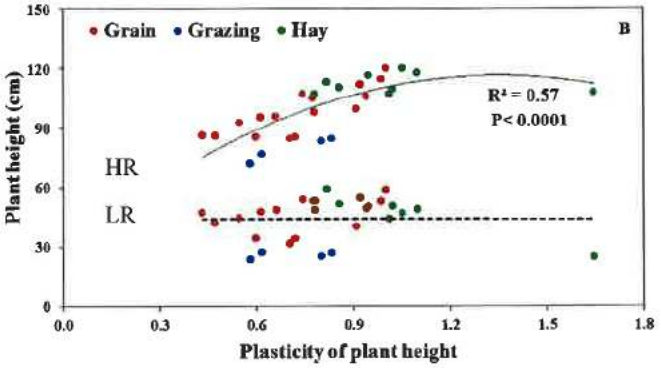


Figure 4 Responsiveness of genotypes to plant height under HR & LR conditions

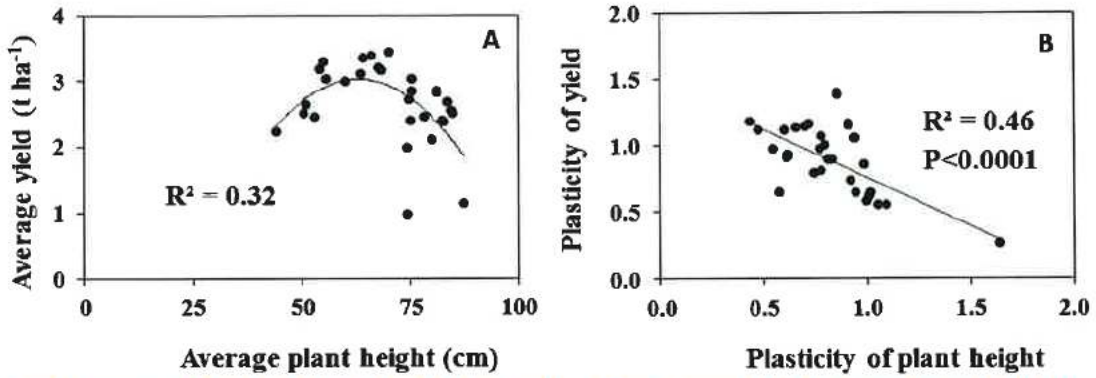


Figure 5 Association between (A) average GY & PH and (B) responsiveness to GY & responsiveness to PH across the enironments

Plant population (Est) , Tiller counts (TC) at SE and HC

Plant population (Est) and per cent tiller difference between HC and harvest were significantly affected by E, G & G x E (Table 5). Responsiveness of the varieties to the LR and HR conditions were equally significant. Correlation analysis of GY with the above traits showed Est influenced GY significantly while per cent reduction in tiller count between SE & HC and HC & harvest did not positively influence GY. Est around six to seven weeks after sowing could be used to predict the yield of a given variety and seems useful than TC and PH

Table 9 Correlation between GY and agonomic traits

	GY	PH	Est	TC_HC-SE	TC_HAR-HC
GY	1.0				
PH	0.1	1.0			
Est	0.6	0.3	1.0		
TC_HC-SE	0.0	0.2	0.1	1.0	
TC_HAR-HC	0.0	-0.5	-0.1	-0.4	1.0

P values	0.001	0.01	0.05

SPAD

All the four SPAD observations (booting as SPAD 1 (Zadoks 41-50), panicle emergence as SPAD 2 (Zadoks 51-60), flowering as SPAD 3 (Zadoks 61-70) and milk development as SPAD 4 (Zadoks 71-77)) were significantly influenced by E, G & G x E sources of variation. The responsiveness of the genotypes to the environment as analysed by VR/ plasticity, showed only low strength of correlation existing both LR & HR locations as indicated by the R2 values in Table 10. Detail analysis of correlation of SPAD vs GY are presented in Table 11 shows that SPAD at LR locations are negatively correlated with the responsiveness (VR) of the variety in LR location while the strength of correlation remains more or less the same under HR conditions.

Table 10 Responsiveness of genotypes to SPAD at different stages to LR & HR locations

Traits	LR		HR	
	P values	R2	P values	R2
Booting	NS	-	0.0075	0.236
Panicle emergence	0.0403	0.147	0.0163	0.196
Flowering	0.0298	0.163	0.0063	0.245
Milk development	0.0022	0.298	NS	-

Table 11 correlation of SPAD with GY

	GY_ VR	GY_ 10	GY_ 90	SPAD 1_VR	SPAD 1_10	SPAD 1_90	SPAD 2_VR	SPAD 2_10	SPAD 2_90	SPAD 3_VR	SPAD 3_10	SPAD 3_90	SPAD 4_VR	SPAD 4_10	SPA 4_9(
GY_VR	1.0														
GY_10	-0.1	1.0													
GY_90	0.6	0.*	1.0												
SPAD1_ VR	0.2	-0.1	0.1	1.0											
SPAD1_ 10	0.2	0.3	0.4	-0.2	1.0										
SPAD1_ 90	0.3	0.2	0.4	0.5	0.*	1.0									
SPAD2_ VR	-0.2	0.0	-0.1	-0.3	0.2	0.0	1.0								
SPAD2_ 10	0.3	0.2	0.3	0.3	0.6	0.*	-0.4	1.0							
SPAD2_ 90	0.1	0.0	0.2	0.0	0.8	0.*	0.4	0.6	1.0						
SPAD3_ VR	0.1	0.0	0.1	-0.3	0.2	0.1	0.5	0.0	0.4	1.0					
SPAD3_ 10	0.1	0.1	0.2	0.3	0.5	0.6	-0.3	0.*	0.4	-0.4	1.0				
SPAD3_ 90	0.2	0.2	0.3	0.0	0.6	0.6	0.1	0.*	0.*	0.5	0.6	1.0			
SPAD4_ VR	-0.1	0.3	0.2	-0.2	0.1	0.1	0.3	-0.1	0.2	0.6	-0.4	0.2	1.0		
SPAD4_ 10	0.2	-0.1	0.1	0.2	0.6	0.6	-0.3	0.*	0.4	-0.2	0.7	0.5	-0.5	1.0	
SPAD4_ 90	0.2	0.1	0.3	0.2	0.7	0.8	0.0	0.*	0.7	0.3	0.5	0.7	0.4	0.6	1

P values	0.001	0.01	0.05
		SPAD_10	
		SPAD_90	

Interesting results were found when analysing the mean SPAD values of all the varieties across the environments for the four stages in relation to GY (Table 12) showed significant positive correlation of SPAD with the GY at all stages of observation with an increase in the strength of correlation indicating the greenness of the leaves at anthesis and milk development is more related to GY than the stages earlier (Figure 6).

Table 12 Correlation of mean SAPD with GY

	GY	Booting	Panicle Emergence	Anthesis	Milk Development
GY	1				
Booting	0.3	1			
Panicle Emergence	0.4	0.8	1		
Anthesis	0.5	0.8	0.9	1	
Milk Development	0.5	0.7	0.8	0.8	1
P value	0.001	0.01	0.05		

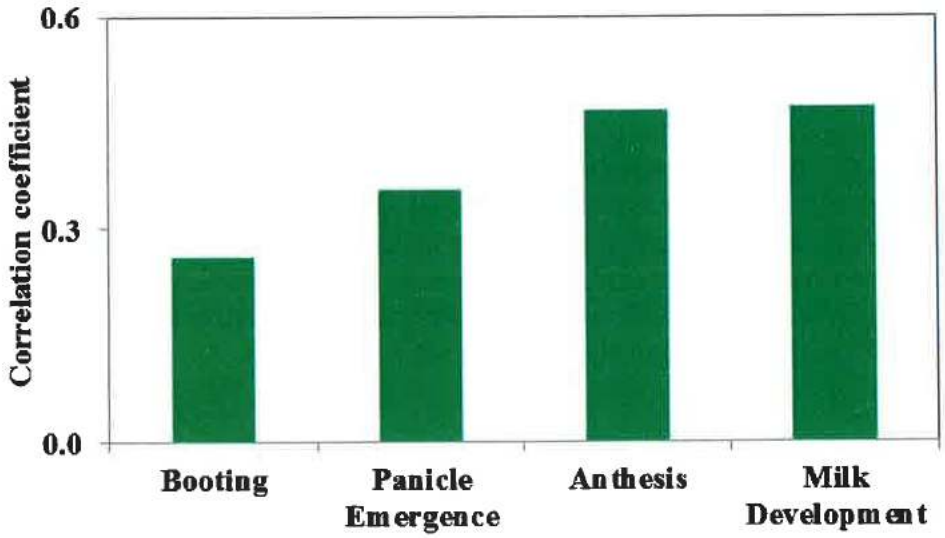


Figure 6 Correlation of SPAD at different stages with GY

Canopy Temperature

Thermal images were captured during designated stages (refer materials and methods). Though the varieties responded significantly to the canopy temperature, there were no meaning full correlation with GY were found. Hence further interpretation of the data was not possible.

Greenseeker

ET and WUE

Evapotranspiration (ET) was significantly influenced by E, but not by G or G x E (Table 5) with higher ET in HR and lower ET in LR regions. BWUE and GWUE were influenced significantly by all the three sources of variation. There was significant responsiveness of the varieties to the environment at both the environments (**Error! Reference source not found.**) but had a negative correlation at LR and positive HR regions (Table..). The genotypes response to BWUE and GWUE were significant and strongly correlated in HR regions only (Figure 7) with no differences in biomass or grain WUE in LR regions. Grazing and hay types generally had lower BWUE and GWUE with lower responsiveness compared to grain types which were efficient in biomass or grain production under favourable conditions with positive contribution. Highest responsiveness (VR) for BWUE and GWUE was observed in 05014-22 (2.37 for both) followed by 05089-31 (2.34 for BUWE & 1.67 for GWUE) and Williams (1.92 for BWUE) and Bannister (1.54) for GWUE.

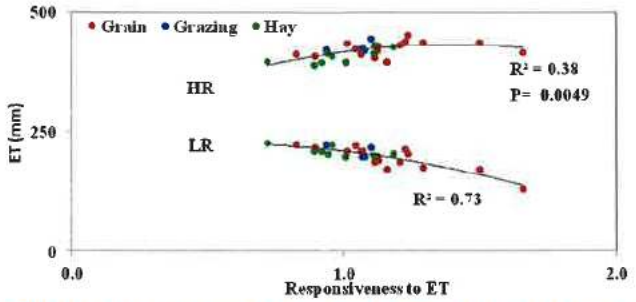
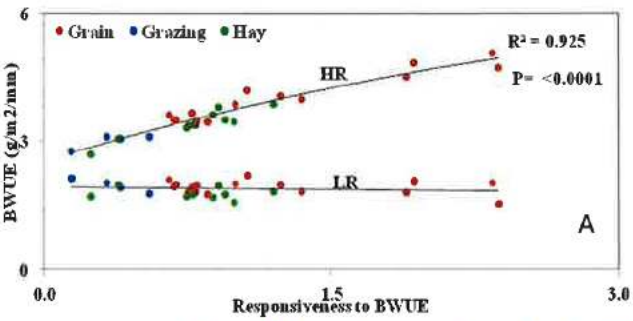


Figure 8 Responsiveness of Genotypes to ET at HR and LR regions



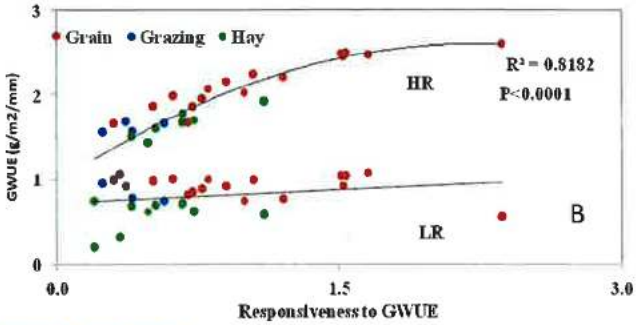
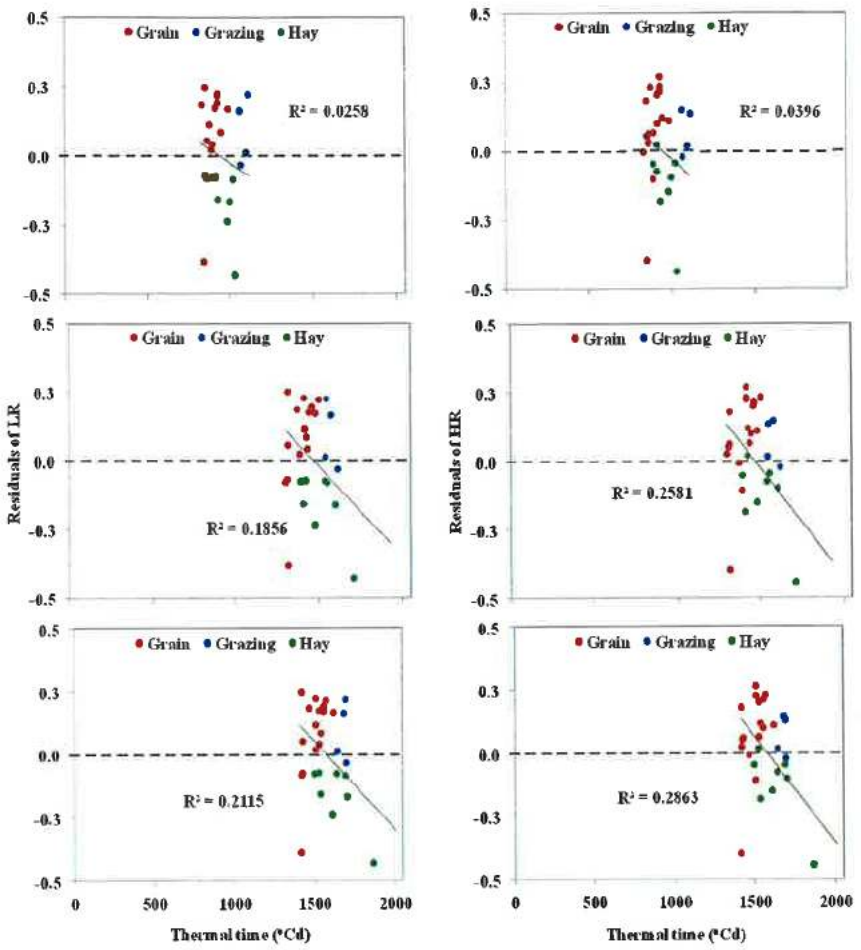


Figure 7 Responsiveness of genotypes to (A) BWUE & (B) GWUE



Residual analysis for ET & BWUE showed no significance while residuals were significant for GWUE. The grain lines, 05014-22 & 05089-31 had high responsiveness but they negatively contributed (0.39 & 0.01 respectively) for GWUE while Mitika had the highest positive residuals (0.27) for GWUE followed by 05089-37 (0.23). GWUE also correlated with the growing degree days to FL and HC at both HR & LR showing differences in response to GWUE between early and late types. The results were similar to GY with late types become less efficient in using water for grain production (Figure 9).

Figure 9 Relationship between residuals of GWUE vs responsiveness at LR & HR environments of genotypes growing degree days from sowing to SE, FL and HC.

BWUE and GWUE were not significant in LR. However, in HR there were significant differences between varieties/lines. The highest BWUE was observed in 05014-22 and

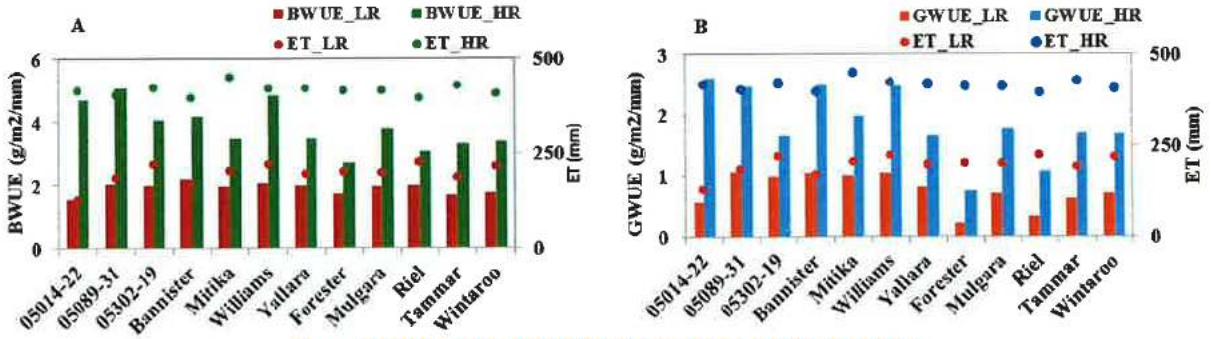


Figure 10 BWUE (A) & GWUE(B) of genotypes at LR & HR regions

Williams Figure 10A. The highest GWUE was 05014-22 followed by Bannister, Williams, and 05089-31 Figure 10B.

Figure 11A shows the grain water use efficiency (GWUE) and Figure 11B shows the biomass water use efficiency (BWUE) of the varieties/lines and ET at four site/years. There is little variation for ET by variety within an environment, but the environments cluster separately,

indicating the significance of the environment.

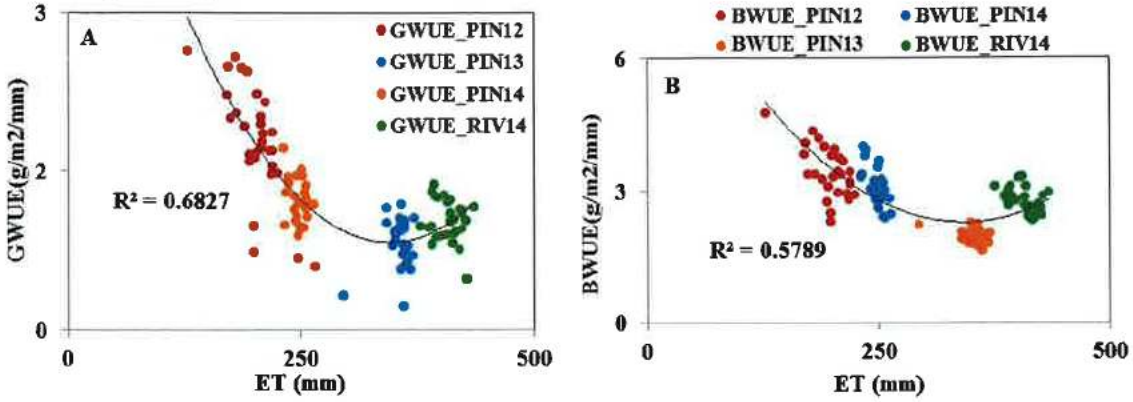
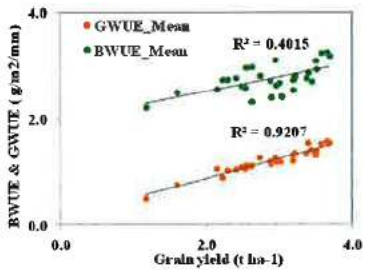


Figure 11 ET response of genotypes to environment vs GWUE (A) & BWUE (B)



The mean BWUE & GWUE of the genotypes correlated with the mean GY of the genotypes with GWUE having stronger correlation.

Entry	Name	Pedigree	Comment
1	11054WO	MITIKA/ IBERIAN-1174	
2	11176WO	04192-2/ IBERIAN-3076	
3	11178WO	YALLARA/ IBERIAN-3011	
4	11179WO	MITIKA/CC7209	
5	11180WO	MITIKA/IBERIAN-32	
6	11181WO	01164-35/CC7216	
7	11184WO	04203-40/IBERIAN-3151	
8	11185WO	YALLARA/ IBERIAN-321	
9	11186WO	03021-42/IBERIAN-3096	
10	11188WO	03021-42/CC7205	
11	11189WO	04136-31/IBERIAN-3019	
12	11192WO	04192-2/IBERIAN-24	
13	11206WO	WAOAT2354-SEL/IBERIAN-3076	
14	11209WO	04200-51/IBERIAN-3037	
15	11211WO	01164-35/IBERIAN-2156	
16	11212WO	WAOAT2332-SEL/IBERIAN-658	
17	11213WO	04203-18/IBERIAN-41	
18	11221WO	04136-31/CC7207	
19	11222WO	04290-3/IBERIAN-3053	
20	11223WO	03122-3/IBERIAN-1427	
21	11247WO	03014-1/IBERIAN-605	
22	11257WO	03122-3/CC7212	Too late for 2012 trial
23	11258WO	04290-3/CC7204	No seed
24	11259WO	FL03007-L1/IBERIAN-3282	
25	11260WO	MN06213-IBERIAN-30	Too late for 1012 trial
26	1126WO	ND040196-CC7208	

The entries highlighted above were promoted in 2013

S12/07: Enhancing the grain yield and quality of oat under water deficits

Final report to SAGIT

August 2015

Dr Pamela Zwer

Dr Mahalakshmi Mahadevan

Dr. Victor Sadras

South Australian Research and Development Institute

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Introduction

The SAGIT approved project on ENHANCING THE GRAIN YIELD AND QUALITY OF OAT UNDER WATER DEFICITS started in July 2012 as a three years project with the following objectives

- Identify traits that will improve the adaptation of oat to water limiting environments in South Australia and Australia allowing for more reliable production in dry seasons and regions.
- Develop and test practical phenotyping techniques.
- Introduce genetic variation using wild oat accessions in the National Oat Breeding Program's germplasm collection.
- Identify parents to create new mapping populations for in-depth genetic studies on adaptation to water deficit.

Project Staff

The project was instigated by Dr. Pamela Zwer, Principal Plant Breeder, SARDI as principal investigator based at the Waite. Dr. Victor Sadras, Climate Applications, SARDI, was involved in the project for the drought tolerance aspects of the project. Dr. Mahalakshmi Mahadevan was appointed as Research Officer for the conduct of the trial.

Other staff members of the National Oat Breeding Program involved in the successful conduct and execution of the research trials were Sue Hoppo, Mark Hill, Kerry Lee, Peter Wheeler and Michelle Williams

Materials and Methods

Test environments and experimental layout

Twenty nine oat entries were chosen for evaluation over three crop seasons (2012, 2013 & 2014) in three different locations each season in Lower North and Mid North regions of South Australia. The entries evaluated consisted of advanced breeding lines, released varieties of grain, hay and grazing types which varied in height, growth habit and maturity **Table 1**. Crops were sown in randomised complete block design with three replications.

A trial environment was a combination of year and location. The environments differed widely for the meteorological conditions and were considered to be high, medium and low rain fall regions based on mean annual rainfall. Details of the environments are provided in **Table 2**. The environments differed widely for the meteorological conditions and were considered to be high, medium and low rain fall regions based on mean annual rainfall.

Plot size was 4.16m^2 (3.2 m x 1.3m) for Pinery, Turretfield and Riverton sites during all the years. Sowing was taken at a seed rate of 165 seeds / m2 with five rows of crop at 0.22m spacing in each plot. Crops were fertilized with 120kg/ha of diammonium phosphate. Plots size at Waikerie was 7.2 m^2 (5 m x 1.44 m), with six rows spaced at 0.254m. Seed rate was 180 seeds /m^2. All other agronomic practices, seed treatment, fertilizer, pest management and herbicide application were carried out in accordance to the specific requirements of each environment, except for disease management.

Four entries, Euro, Glider, Kangaroo and Mortlock (highlighted in **Table** 1)were not uniform for all the trial sites, hence were disregarded for statistical analysis to keep the genotypes

uniform between the environments. Details of different traits observed are detailed in (A & B)

Observations

Soil sampling

Initial soil samples at sowing were collected using a mechanically driven soil rig at 20cm intervals up to one meter depth. Soil moisture was estimated by gravimetric method and expressed as volumetric water content (mm). Soils were analysed for soil characteristics (by CSBP, WA); EC, pH, Boron, sodium, nitrate nitrogen, ammonical nitrogen, bulk density, soil type and classification. Final soil samples were collected by adopting the same procedure for each plot representing one genotype, to a depth of 1m to estimate volumetric soil moisture content (mm). Evapotranspiration (ET) of individual genotypes was estimated from the initial and final soil moisture content and the amount of rainfall received from sowing to harvest. ET was used to estimate Water Use Efficiencies (WUE); biomass water use efficiency (BWUE) and grain water use efficiency (GWUE)

Phenology: The crop growth stages were scored at weekly intervals using Zadoks scales to estimate days taken to and thermal degree days to the critical stages; stem elongation (SE), flowering (FL) and hay cutting stage (HC)

Establishment: plant population was counted around six weeks after sowing and were expressed as numbers per m^2

Tiller count: Tiler counts were at SE (Zadoks 31) and HC (Zadoks 71) and expressed as number per m^2

Greenseeker: Greenseeker (Greenseeker Hand Held optical Sensor Unit, model 505, NTech Industries, Inc. California, USA) was used to measure the NDVI (Normalised Difference Vegetative Index) of individual lines at fortnightly intervals from eight weeks after sowing at fortnightly intervals through to harvest during 2014 season and until a point when NDVI values started to decline during 2014 season. These observations where used to project the biomass of the genotype from the regression equation obtained from the calibration of NDVI and biomass of selected lines.

Canopy temperature: Thermal images were captured using FLIR B365 camera with 25 °C during clear still day days, preferably between 11 am to 2 pm and further processed using FLIR quick reporter software. Four crop growth stages were selected to capture the images: half panicle emergence (Zadoks 55), panicle emergence completed (Zadoks 59), flowering (Zadoks 60 – 65) and grain development (Zadoks 70-77). There were practical difficulties in capturing images at the right stage of crop growth during 2013 season. However, they were successfully captured during 2014 season. Five images were captured in Pinery and six in Riverton while only three and four images were used for the sites respectively since all varieties were captured on the same day.

SPAD: Handheld Chlorophyll meter SPAD502 Plus, measures the relative amount of chlorophyll present in the leaves, which served as an overall indicator of plant health and "stay green status". SPAD values of individual plots were recorded in flag leaf at four important growth stages, booting as SPAD 1 (Zadoks 41-50), panicle emergence as SPAD 2

(Zadoks 51-60), flowering as SPAD 3 (Zadoks 61-70) and milk development as SPAD 4 (Zadoks 71-77).

Hay yield: Biomass cuts from 0.5m length of the centre three rows (total of 1.5 m) were taken from individual plots during stage milk development stage (Zadoks 71) and hay yields were estimated and expressed as g/m2. Biomass cuts of 0.5 m lengths from two border rows (making up 1m length in total) were taken separately to the centre rows to estimate the yield parameters only. This was done for one of the locations, Riverton. The biomass was collected in individual paper bags, labelled and subsequently dried in the oven at 50-60˚C in the oven for 3 days to record dry weight. Subsequently biomass per m^2 would be determined.

Plant height: Plant height was measured at physiological maturity (before harvest).

Final biomass: Quadrant samples (0.5m x 3 rows) were collected from individual plots (genotypes) to estimate total biomass.

Yield and Quality: The quadrant samples collected for biomass estimation were used to determine yield and yield components, grain physical and NIR qualities.

The traits observed &/ computed, abbreviations and the units they are expressed are listed in Table 4

Table 1. List and description of oat genotypes used for the study

Entry no.	Name	Type	Height	Growth habit	Maturity	Greenseeker calibration variety
1	Bannister	Grain	Tall Dwarf	Semi-erect	Mid	W
2	Bettong	Hay	Medium Tall	Semi-erect	Mid	Y
3	Brusher	Hay	Tall	Semi-erect	Early-mid	Y
4	Carrolup	Hay	Medium Tall	Semi-erect	Early-mid	Y
5	Dunnart	Grain	Tall Dwarf	Semi-erect	Early-mid	W/M
6	Echidna	Grain	Dwarf	Semi-erect	Mid	M
7	Eurabbie	Grazing	Dwarf	Semi-erect	Mid-late	MA
8	Euro	Grain	Medium Tall	Erect	Early-mid	NOT USED
9	Forester	Hay	Tall	Semi-erect	Late	F
10	Glider	Hay	Medium Tall	Semi-erect	Late	G
11	Kangaroo	Hay	Medium Tall	Semi-erect	Mid-late	G
12	Mitika	Grain	Dwarf	Semi-erect	Early	M
13	Mortlock	Grain	Medium Tall	Semi-erect	Early-mid	Y
14	Mulgara	Hay	Tall	Semi-erect	Mid	Y
15	Potoroo	Grain	Tall Dwarf	Semi-erect	Early-mid	W/M
16	Riel	Hay	Medium Tall	Semi-erect	Late	F
17	Tammar	Hay	Medium Tall	Semi-erect	Mid-late	Y
18	Tungoo	Hay	Tall	Erect	Mid-late	Y
19	Wallaroo	Hay	Tall	Semi-erect	Early	Y
20	Williams	Grain	Medium Tall	Semi-erect	Early-mid	W
21	Wintaroo	Hay	Tall	Semi-erect	Mid	Y
22	Wombat	Grain	Dwarf	Semi-erect	Mid	M
23	Yallara	Grain	Medium Tall	Erect	Early-mid	Y
24	MA6875	Grazing	Dwarf	Prostrate	Mid-late	MA
25	MA7930	Grazing	Dwarf	Prostrate	Mid-late	MA
26	MA9345	Grazing	Dwarf	Prostrate	Mid-late	MA
27	05014-22	Grain	Tall	Semi-erect	Early	Y
28	05089-31	Grain	Tall Dwarf	Semi-erect	Mid	W/M
29	05089-37	Grain	Tall Dwarf	Semi-erect	Early	W/M
30	05097-17	Grain	Medium Tall	Erect	Early	Y
31	05104-19	Grain	Tall	Erect	Early	Y
32	05140-3	Grain	Medium Tall	Erect	Very early	Y
33	05302-19	Grain	Medium Tall	Erect	Early	Y

Lines used for greenseeker calibration
MA6875 designated as MA
Yallara designated as Y
Forester designated as F
Glider designated as G
Mitika designated as M
Williams designated as W

Table 2. Details of the trial environments (season & location) selected for evaluation of 29 oat entries

Season	Location	Environment	Rainfall zone	T_{Max} (℃)	T_{Min}(℃)	Rainfall during the growing season (mm)	Date of sowing	GPS Coordinates	Meteorological station (No.)	Evapotranspiration during the growing period (mm)
2012	Turretfield	TRC12	Medium	11.0 to 40.6	-1.0 to 25.5	236.6	8th June	34°32′ 35.38″S 138°49′ 32.46″E	Rosedale (23343)	606.1
	Pinery	PIN12	Low	12.0 to 40.0	-0.5 to 24.5	199.8	5th June	34°19′ 44.48″S 138°28′ 51.98″E	Owen (23012)	549.3
	Waikerie	WAK12	Low	12.5 to 39.5	-4.5 to 25.0	75.3	30th May	34°15′ 19.8″S 140°0′ 08″E	Waikerie (24018)	486.6
2013	Riverton	RIV13	High	11.0 to 40.0	0.5 to 20.5	365.9	29th May	34° 12′ 01.30″ S 138° 44′ 24.29″ E	Riverton (23314)	547.0
	Turretfield	TRC13	Medium	10.7 to 39.8	0.4 to 24.8	262.1	25th June	34°32′ 36.20″ S 138°49′19.53″ E	Rosedale (23343)	512.3
	Pinery	PIN13	Low	11.5 to 38.0	1.0 to 17.0	257.1	28th May	34°19′ 24.27″ S 138°29′14.00″ E	Owen (23012)	423.7
2014	Riverton	RIV14	High	11.0 to 41.0	-1.5 to 22.5	282.7	30th May	34° 13′ 11.13″ S 138° 44′ 3.08″ E	Riverton (23314)	627.6
	Turretfield	TRC14	Medium	10.5 to 41.4	-1.4 to 24.2	251.8	6th June	34°32′ 59.10″S 138°50′ 28.30″E	Rosedale (23343)	525.4
	Pinery	PIN14	Low	11.5 to 41.5	-1.0 to 22.5	174.0	26th May	34°20′ 39.90″ S 138°29′23.65″ E	Owen (23012)	526.0

Table 3. Trial location, season, experiment details and various traits observed

Season	Location	Environment	Experiment		Soil sampling			Agronomic traits						
			Number of entries	Number of replications	Initial	Final	EM mapping	plant population /m2	Tiller Count /m2	Tiller Count /m2 (GS71)	Phenology	Plant Height (cm)	SPAD	Canopy temp
2012	PIN	PIN12	32	3	√	√	×	×	×	×	×	√	√ (1)	×
	TRC	TRC12	30	2	×	×	×	×	×	×	×	×	√ (1)	×
	WAK	WAK12	30	3	×	×	×	×	×	×	×	√	×	×
2013	PIN	PIN13	32	3	√	√	√	√	√ (60 DAS)	√	√	×	√ (4)	×
	RIV	RIV13	32	3	√	×	√	√	√ (60 DAS)	√	√	×	√ (4)	×
	TRC	TRC13	32	3	√	×	×	×	×	×	×	×	×	×
2014	PIN	PIN14	32	3	√	√	√	√	√ (GS 31)	√	√	√	√ (5)	√ (8)
	RIV	RIV14	32	3	√	√	√	√	√ (GS 31)	√	√	√	√ (6)	√ (8)
	TRC	TRC14	32	3	√	×	×	×	×	×	×	×	×	×
No. of environments					7	4	4	4	4	4	4	4	4	2

Table 3 (Conti.) Trial location, season, experiment details and various traits observed

Season	Location	Environment	Hay		Final biomass		Yield & yield components						Grain Quality	
			Yield	NIR	Weight (g/m2)	NIR	Grain Yield	Grain Number	Grain Size	Number of heads	Number of grains /head	Head weight	Physical	NIR
2012	PIN	PIN12	√	√	√	√	√	√	√	√	√	√	√	√
	TRC	TRC12	×	×	×	×	√	√	√	×	×	×	√	√
	WAK	WAK12	×	×	×	×	√	×	×	×	×	×	×	×
2013	PIN	PIN13	√	√	√	√	√	√	√	√	√	√	√	√
	RIV	RIV13	√	√	√	√	√	√	√	√	√	√	√	√
	TRC	TRC13	×	×	×	×	√	×	×	×	×	×	×	√
2014	PIN	PIN14	√	√	√	√	√	√	√	√	√	√	√	√
	RIV	RIV14	√	√	√	√	√	√	√	√	√	√	√	√
	TRC	TRC14	×	×	×	×	√	√	√	×	×	×	√	√
No. of environments			5	5	5	5	5	5	7	5	5	5	7	8

Table 4. Abbreviations and units of traits measured &/ computed

Traits measured &/ computed	Abbreviations	Units
Water Use Efficiency	WUE	
Evapotranspiration	ET	Mm
Biomass water use efficiency	BWUE	
Grain water use efficiency	GWUE	
Plant population	EST	Number /m2
Tiller counts	TC	Number /m2
Plant Phenology scores		Zadoks scale
Days to SE (Zadoks 31)	Days_SE	Number
Days to FL (Zadoks 60)	Days_FL	Number
Days to HC (Zadoks 71)	Days_HC	Number
Thermal degree days to SE (Zadoks 31)	Cd_SE	Degree days
Thermal degree days to FL (Zadoks 60)	Cd_FL	Degree days
Thermal degree days to HC (Zadoks 71)	Cd_HC	Degree days
Hay yield	HY	g/m2
Crude protein	HCP	%
Water soluable carbohydrates	HWSC	%
Nitrogen	HN	%
Digestibility	Hdig	%
Metabolisable energy	HME	%
Acid detergent fiber	HADF	%
Neutral detergent fiber	HNDF	%
SPAD	SPAD	value
NDVI	NDVI	value
Canopy temperature	CT	◦c
Plant height	PH	Cm
Yield & Yield components		
Grain Yield	GY	t/ha
Grain number	GN	Number
Number of heads	NOH	Number
Head weight	HW	g/m2
Number of grains per head	NOG/H	Number
Grain size(1000 grain weight)	GS	G
Hectolitre weight	HLW	Kg
screening	SCR	%
Protein	PRO	%
Oil	OIL	%
Groat	GRO	%
β-Glucan	βGlu	%
Biomass at harvest	BHAR	g/m2
Harvest Index	HI	
Dry matter	SDM	%
Straw Crude protein	SCP	%

Straw Water soluable carbohydrates	SWSC	%
Straw Nitrogen	SN	%
Straw Digestibility	Sdig	%
Straw Acid detergent fiber	SADF	%
Straw Neutral detergent fiber	SNDF	%

Results and discussion:

All the traits observed (**Table** 3. A &B) and those computed from primary observations (Table 4) were statistically analysed to estimate the traits for phenotypic variance; Environment (E), Genotype (G) and G x E interaction. All the above three sources of variation had significant influence on most of the traits with P values < 0.05. This indicated the diversity of the genotypes and the environments selected for the study. It also allowed us to study and understand the response (**plasticity**) or behaviour of the genotypes to the environment which were determined statistically through variance ratio (**VR**). The responsiveness of a trait was environment dependent. The strength of correlation of VR (trait plasticity) as indicated by the R^2 values, was the strongest in the high rainfall (HR) (90^{th} percentile) and weakest in the low rainfall (LR) (10^{th} percentile). Higher the VR, higher was the responsiveness of a genotype to the environment. Regression and residual analysis of the correlation helped us to study the strength of association between the traits and their responsiveness and if it were positively or negatively contributing towards the trait at HR & LR locations.

Grain yield (GY)

RIV13 registered the highest (365mm) and WAK12 the lowest (75mm) rainfall from sowing to harvest. The difference between the evaporative demand and rainfall was the highest for WAK12 (411mm) and lowest for RIV13 (263). Grain yield was analysed for nine environments. The environment mean yield ranged from 0.3 t/ha at Waikerie (LR) to 4.4 t/ha at Riverton (HR). The top yielding lines Bannister, Mitika, Dunnart, and 05302-19 averaged approximately 3.3 t ha-1 across the environments and the lowest yielding lines were Forester 0.93t/ha, Riel 1.16 t/ha and Tammar 1.95 t/ha.

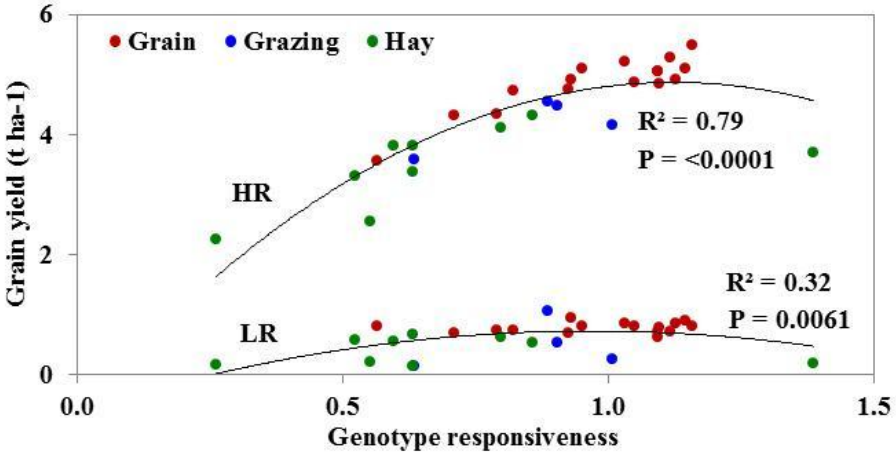


Figure 1. yield response of genotypes to HR and LR conditions

Genotypic differences in responsiveness to grain yield in LR and HR environments are shown in Figure 1. Residual analysis for grain yield indicated milling varieties produce above average yields (red dots) and hay and grazing varieties produced below average yields (green and blue dots). The late hay variety, Forester, was the least responsive variety, 0.26, for grain yield, producing 0.16 t/ha in low rainfall (LR) environment, increasing to 2.25 t/ha in high rainfall (HR). The milling variety, Bannister, had the highest responsiveness of 1.16 producing 0.80 t/ha in LR, but increasing to 5.47 t/ha in HR. The milling variety, Mitika, had the next highest response of 1.12 producing 0.71 t/ha in LR increasing to 5.29 t/ha in HR. The high responsiveness indicates the varieties produced slightly higher than the average grain yield in LR, but could take advantage of HR environments and produce higher than average grain yields.

Significant differences were observed in growing degree days (Cd) from sowing to stem elongation (SE), flowering (FL), and hay cut (HC) between the varieties/lines (G) and for SE & FL between the environments, but not for HC or GxE (Table 5). Grain yield in both HR and LR conditions significantly correlated with growing degree days to SE, FL, and HC. The relationship showed that the varieties/lines which attained SE before 894°Cd, FL before 1683°Cd and HC earlier than 1861°Cd produced above average yields under favorable environments (Figure 2). Similar thresholds were found for the stressful conditions.

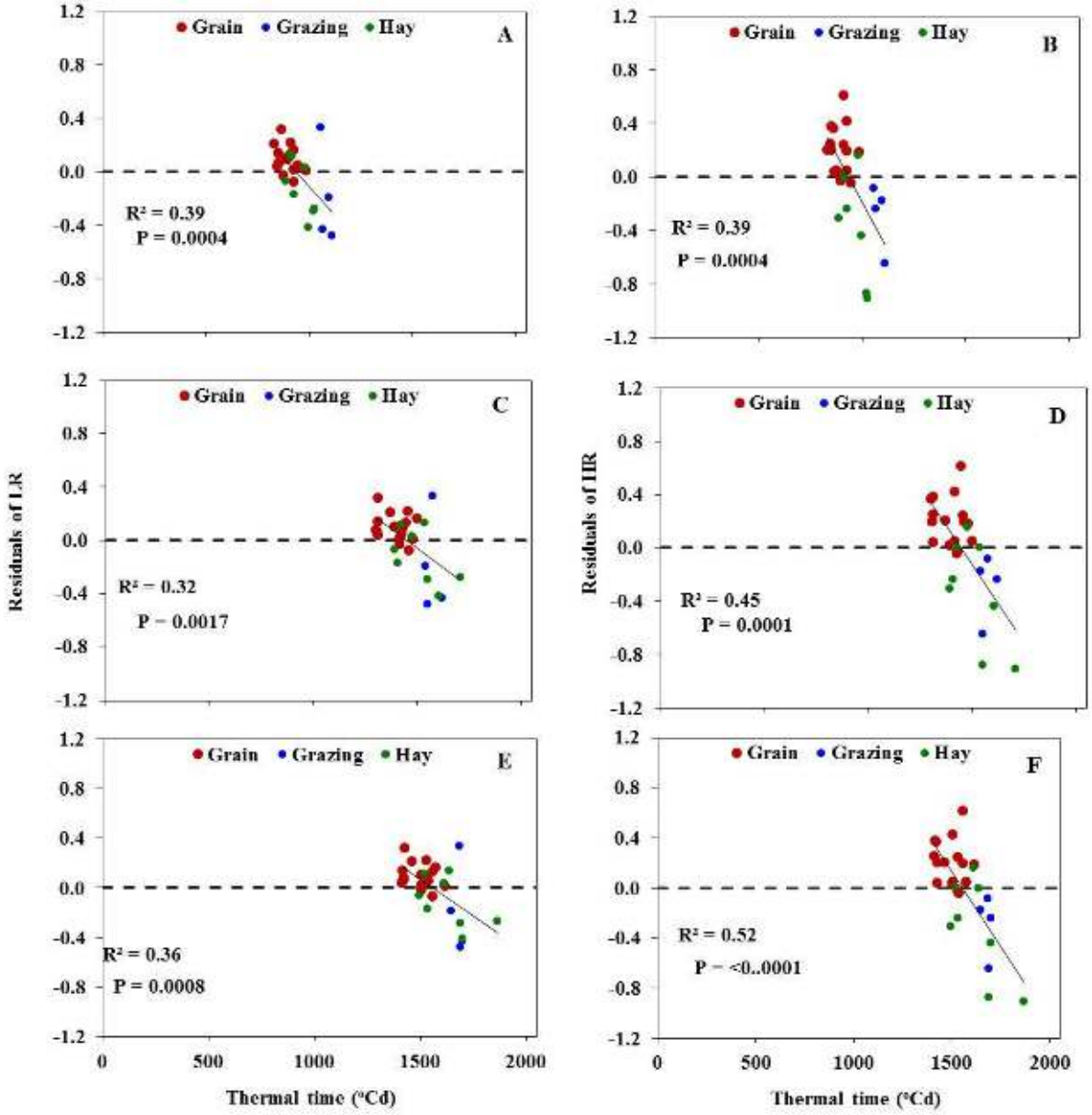


Figure 2. Relationships between residuals of yield vs responsiveness at LR and HR environments of 29 entry's growing degree days from sowing to SE (A & B), FL (C & D) and HC (E & F). Forester, an extremely late variety was excluded from the analysis

Yield components (GN, GS, NOH & NOG/H)

ANOVA for yield components; GN, GS, NOH & NOG/H (Table 5) showed highly significant influence of E, G and G x E for all the above components (P <0.0001). Yield components were analysed for responsiveness (plasticity/VR) of genotypes to LR and HR locations and presented in Table 6. Responsiveness of yield components & grain physical and NIT qualities to LR and HR. GN, NOH & NOG/H had very highly significant correlation with HR

locations and were non-significant (NS) at LR locations. While, GS was exactly opposite, significantly correlated at LR and NS for HR locations. This means that owing to poor number of grain set, low number of heads and less number of grains per head (**SINK**) under LR conditions favoured better GS.

Table 5. P values of ANOVA for E, G, G x G for various traits

	GY (9 E)	GY (7E)	GN (7E)	GS (7E)	NOH (5E)	NOG/H (5E)	HLW (5E)	SCR % (5E)	Protein % (8E)	Oil % (8E)	Groat % (8E)	β-Glucan (8E)
	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value
Environment (E)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Genotype (G)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
G x E	**<0.0001**	**<0.0001**	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

	HI (5E)	HY (5E)	HWSC (5E)	HN (5E)	Hdig (5E)	HME	HADF	HNDF	SWSC (5E)	SN (5E)	Sdig	SSDF	SNDF
	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value
Environment (E)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Genotype (G)	**<0.0001**	**<0.0001**	**<0.0001**	**0.0067**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
G x E	**<0.0001**	**0.0122**	**<0.0001**	**0.0046**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**NS**	**0.0001**	**<0.0001**	**<0.0001**

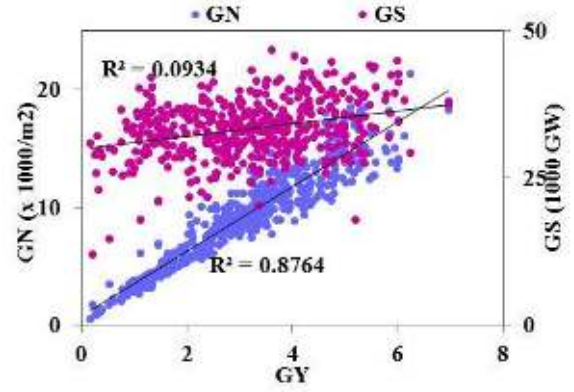
	PH (4E)	Est (4E)	Tillers: GS 71 & GS31 (2E)	Tillers: GS71 & harvest (4E)	Cd_SE (4E)	Cd_FL (4E)	Cd_HC (4E)	Cd_SE (4E)	Cd_FL (4E)	Cd_HC (4E)	Days_SE (4E)	Days_FL (4E)	Days_HC (4E)	Days_SE (4E)	Days_FL (4E)	Days_HC (4E)
	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value
Environment (E)	**<0.0001**	**<0.0001**	**NS**	**<0.0001**	**<0.0001**	**0.0055**	**NS**				**<0.0001**	**<0.0001**	**<0.0001**			
Genotype (G)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**				**<0.0001**	**<0.0001**	**<0.0001**				**<0.0001**	**<0.0001**	**<0.0001**
G x E	**<0.0001**	**<0.0001**	**0.002**	**<0.0001**												

	Cd SE (4E)	Days_SE (4E)	Days_FL (4E)
	P-Value	**P-Value**	**P-Value**
Environment (E)	**<0.0001**	**<0.0001**	**<0.0001**
Genotype (G)	**<0.0001**	**<0.0001**	**<0.0001**
G x E	**<0.0001**	**<0.0001**	**<0.0001**

	SPAD 1 (4E)	SPAD 2 (4 E)	SPAD 3 (4E)	SPAD 4 (4E)	SPAD 5 (2E)	SPAD 6 (1E)	ET (4E)	BWUE (4 E)	GWUE (4E)
	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value	P-Value
Environment (E)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**<0.0001**	**<0.0001**	**<0.0001**
Genotype (G)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**NS**	**<0.0001**	**<0.0001**
G x E	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**		**NS**	**0.0007**	**<0.0001**

Table 6. Responsiveness of yield components & grain physical and NIT qualities to LR and HR

Traits	LR		HR	
	P values	R2	P values	R2
GN	NS	-	<0.0001	0.664
GS	0.0187	0.264	NS	-
NOH	NS	-	0.009	0.304
NOG/H	NS	-	<0.0001	0.795
HLW	0.0001	0.424	0.0214	0.181
SCR	<0.0001	0.444	<0.0001	0.906
PRO	NS	-	0.0004	0.379
OIL	NS	-	0.0007	0.351
GRO	0.0002	0.399	NS	-
βglu	<0.0001	0.627	NS	-



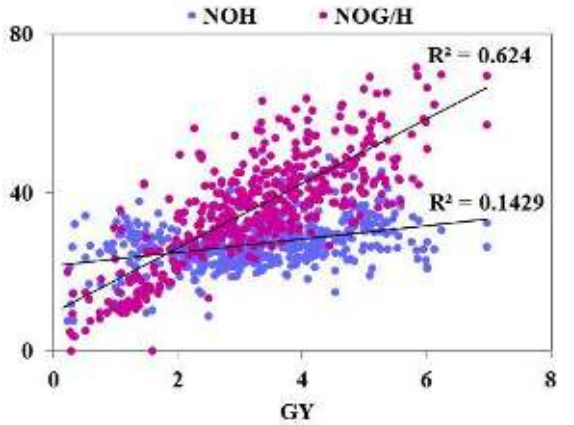


Figure 3. Association of GY with yield components: GN, GS, NOH & NOG/H

Correlation analysis between yield and yield components revealed GN and NOG/H were main contributors for GY than GS and NOH (**Error! Reference source not found.**). Correlation analysis showed zero correlation of GS with GY while GN had the strongest value of 1 (Table 7). A negative correlation relationship was evident between GS & GN and GS & NOG/H, which means higher the GN and NOG/H lower, is the GS.

Grain Quality

E, G & G x E significantly influenced the grain physical and NIR qualities; hectolitre weight (HLW), Screening (SCR), protein (PRO), oil (OIL), groat (GRO) and β glucan (βglu) (Table 5). Responsiveness (plasticity/VR) of genotypes to LR and HR conditions showed significant results for HLW & SCR under both the conditions while significant response was observed for PRO & GRO to HR and GRO & βglu to LR (Table 6). Williams noticeably had the highest SCR among the grain varieties. SCR also had significant negative correlation with GS, HLW and GRO. Similar relationship existed between PRO and HLW & OIL(Table 7).

Table 7. Correlation of yield components and grain physical and NIR qualities with GY

	GY	GN	GS	NOH	NOG/H	HLW	SCR	PRO	OIL	GRO	βGlu
GY	1										
GN	1.0	1									
GS	0.0	-0.1	1								
NOH	0.5	0.5	0.1	1							
NOG/H	0.9	0.9	-0.2	0.1	1						
HLW	0.2	0.0	0.6	0.1	0.0	1					
SCR	0.3	0.4	-0.7	0.0	0.5	-0.5	1				
PRO	-0.2	-0.2	0.0	0.3	-0.4	-0.3	0.0	1			
OIL	-0.1	-0.1	0.1	-0.1	-0.1	0.4	-0.2	-0.4	1		
GRO	0.2	0.1	0.5	0.3	0.1	0.5	-0.4	-0.1	0.2	1	
βGlu	-0.1	-0.1	0.0	0.3	-0.2	-0.1	-0.2	0.3	0.0	0.2	1

P values	0.001	0.01	0.05

Hay yield and quality; Harvest index (HI)

Hay yield was significantly influenced by E, G & G x E (Table 5). Hay yield response to the environment did not show any pattern of response whether the genotypes were grain or hay types. Though hay quality traits were significantly influenced by the E, their response to HR was more pronounced than to LR.

HI (yield divided by total biomass produced) was significantly influenced by the three sources of variation (Table 5) with the responsiveness of genotypes to HR conditions alone being significant. The residual effects were similar to that of GY with grain varieties being positive than hay or grazing types. The reason being HI was determined more by GY than by biomass as evident by the correlation coefficient values in Table 8.

Table 8. Correlation of GY with HI and hay & straw yield and quality

	GY	HI	HY	HWSC	HN	SWSC	SN
GY	1						
HI	0.9	1					
HY	0.5	0.2	1				
HWSC	0.1	0.0	0.3	1			
HN	0.3	0.0	0.8	-0.2	1		
SWSC	0.0	-0.1	0.0	0.8	-0.3	1	
SN	-0.3	-0.5	0.2	-0.2	0.5	-0.3	1

Agronomic traits

Plant height

Plant height was influenced by three sources of variation). Average plant height across environments ranged from 45 to 99 cm. Plasticity of plant height ranged from 0.43 to 1.64

and correlated with HR but not with LR Figure 4. This means plasticity resulted from responsiveness to favourable conditions with no systematic variation among lines under stress. Average yield was non-linearly related to plant height and there was a negative relationship between plasticity of yield and plasticity of height (Figure 5A) Analysis of plant height is vital in breeding as this trait influences harvest index and yield (Figure 5B) with dwarf genotypes possessing greater yield potential than the tall genotypes. However, the tall genotypes have greater potential for hay yield

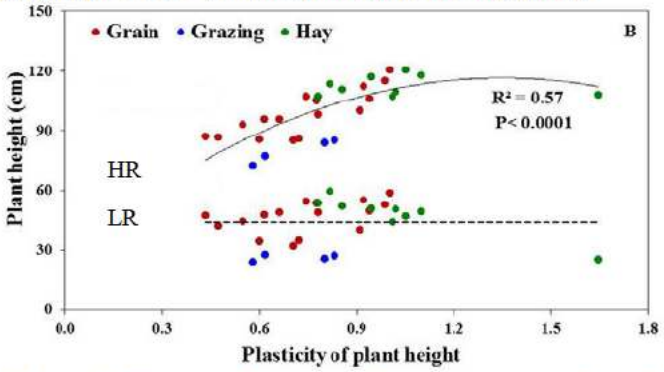


Figure 4. Responsiveness of genotypes to plant height under HR & LR conditions

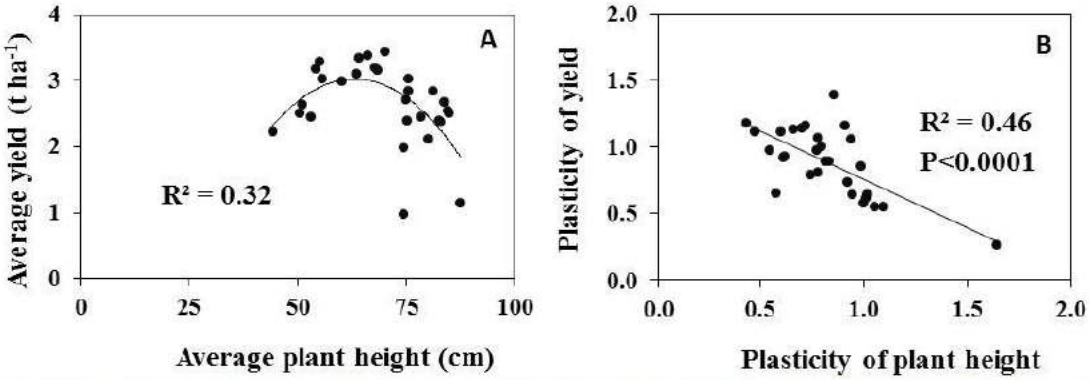


Figure 5. Association between (A) average GY & PH and (B) responsiveness to GY & responsiveness to PH across the enironments

Plant population (Est) , Tiller counts (TC) at SE and HC

Plant population (Est) and per cent tiller difference between HC and harvest were significantly affected by E, G & G x E (Table 5). Responsiveness of the varieties to the LR and HR conditions were equally significant. Correlation analysis of GY with the above traits showed Est influenced GY significantly while per cent reduction in tiller count between SE & HC and HC & harvest did not positively influence GY (Table 9). Est around six to seven weeks after sowing could be used to predict the yield of a given variety and seems useful than TC and PH

Table 9 Correlation between GY and agonomic traits

	GY	PH	Est	TC HC-SE	TC HAR-HC
GY	1.0				
PH	0.1	1.0			
Est	0.6	0.3	1.0		
TC HC-SE	0.0	0.2	0.1	1.0	
TC_HAR-HC	0.0	-0.5	-0.1	-0.4	1.0

P values	0.001	0.01	0.05

SPAD

All the four SPAD observations (booting as SPAD 1 (Zadoks 41-50), panicle emergence as SPAD 2 (Zadoks 51-60), flowering as SPAD 3 (Zadoks 61-70) and milk development as SPAD 4 (Zadoks 71-77)) were significantly influenced by E, G & G x E sources of variation. The responsiveness of the genotypes to the environment as analysed by VR/ plasticity, showed only low strength of correlation existing both LR & HR locations as indicated by the R2 values in Table 10. Detail analysis of correlation of SPAD vs GY are presented in

Table 11 shows that SPAD at LR locations are negatively correlated with the responsiveness (VR) of the variety in LR location while the strength of correlation remains more or less the same under HR conditions.

Table 10. Responsiveness of genotypes to SPAD at different stages to LR & HR locations

Traits	LR		HR	
	P values	R2	P values	R2
Booting	NS	-	0.0075	0.236
Panicle emergence	0.0403	0.147	0.0163	0.196
Flowering	0.0298	0.163	0.0063	0.245
Milk development	0.0022	0.298	NS	-

Table 11. correlation of SPAD with GY

	GY_ VR	GY_ 10	GY_ 90	SPAD 1 VR	SPAD 1 10	SPAD 1 90	SPAD 2 VR	SPAD 2 10	SPAD 2 90	SPAD 3 VR	SPAD 3 10	SPAD 3 90	SPAD 4 VR	SPAD 4 10	SPAD 4 90
GY_VR	1.0														
GY 10	-0.1	1.0													
GY_90	0.6	0.7	1.0												
SPAD1_ VR	0.2	-0.1	0.1	1.0											
SPAD1_ 10	0.2	0.3	0.4	-0.2	1.0										
SPAD1_ 90	0.3	0.2	0.4	0.5	0.7	1.0									
SPAD2_ VR	-0.2	0.0	-0.1	-0.3	0.2	0.0	1.0								
SPAD2_ 10	0.3	0.2	0.3	0.3	0.6	0.7	-0.4	1.0							
SPAD2_ 90	0.1	0.0	0.2	0.0	0.8	0.7	0.4	0.6	1.0						
SPAD3_ VR	0.1	0.0	0.1	-0.3	0.2	0.1	0.5	0.0	0.4	1.0					
SPAD3_ 10	0.1	0.1	0.2	0.3	0.5	0.6	-0.3	0.7	0.4	-0.4	1.0				
SPAD3_ 90	0.2	0.2	0.3	0.0	0.6	0.6	0.1	0.7	0.7	0.5	0.6	1.0			
SPAD4_ VR	-0.1	0.3	0.2	-0.2	0.1	0.1	0.3	-0.1	0.2	0.6	-0.4	0.2	1.0		
SPAD4_ 10	0.2	-0.1	0.1	0.2	0.6	0.6	-0.3	0.7	0.4	-0.2	0.7	0.5	-0.5	1.0	
SPAD4_ 90	0.2	0.1	0.3	0.2	0.7	0.8	0.0	0.7	0.7	0.3	0.5	0.7		0.6	1.0

P values	0.001	0.01	0.05
		SPAD_10	
		SPAD_90	

Interesting results were found when analysing the mean SPAD values of all the varieties across the environments for the four stages in relation to GY (Table 12) showed significant positive correlation of SPAD with the GY at all stages of observation with an increase in the strength of correlation indicating the greenness of the leaves at anthesis and milk development is more related to GY than the stages earlier (Figure 6).

Table 12. Correlation of mean SAPD with GY

	GY	Booting	Panicle Emergence	Anthesis	Milk Development
GY	1				
Booting	0.3	1			
Panicle Emergence	0.4	0.8	1		
Anthesis	0.5	0.8	0.9	1	
Milk Development	0.5	0.7	0.8	0.8	1
P value	0.001	0.01	0.05		

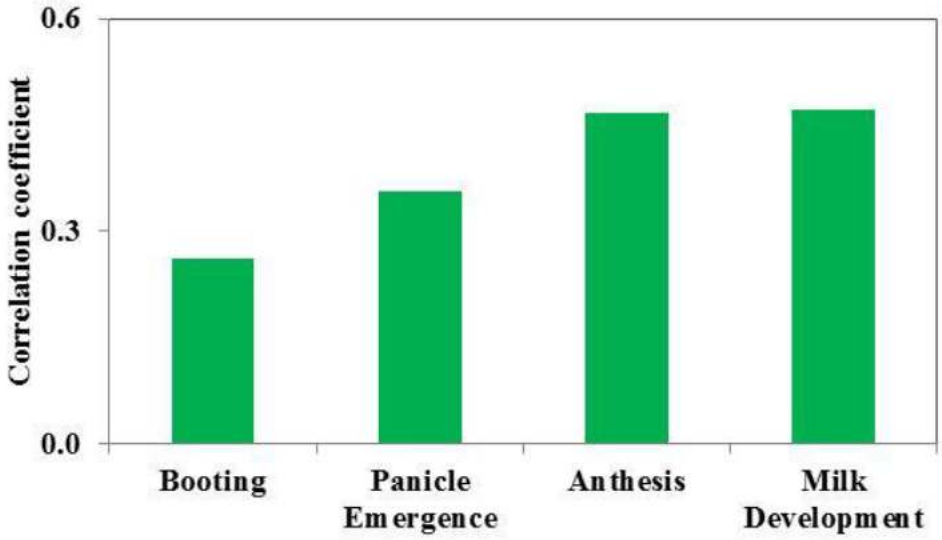


Figure 6. Correlation of SPAD at different stages with GY

Canopy Temperature

Thermal images were captured during designated stages (refer materials and methods). Though the varieties responded significantly to the canopy temperature, there were no meaning full correlation with GY were found. Hence further interpretation of the data was not possible.

ET and WUE

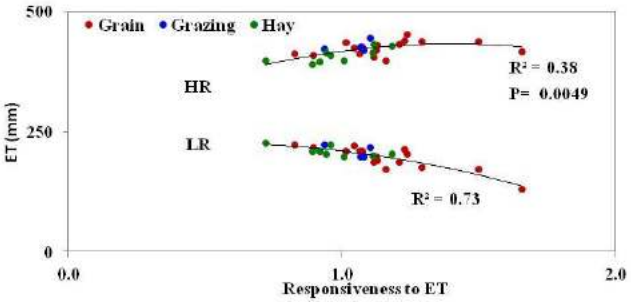


Figure 7. Responsiveness of Genotypes to ET at HR and LR regions

Evapotranspiration (ET) was significantly influenced by E, but not by G or G x E (Table 5) with higher ET in HR and lower ET in LR regions. BWUE and GWUE were influenced significantly by all the three sources of variation. There was significant responsiveness of the varieties to the environment at both the environments (Figure 7) but had a negative correlation at LR and positive HR regions. The genotypes response to BWUE and GWUE were significant and strongly

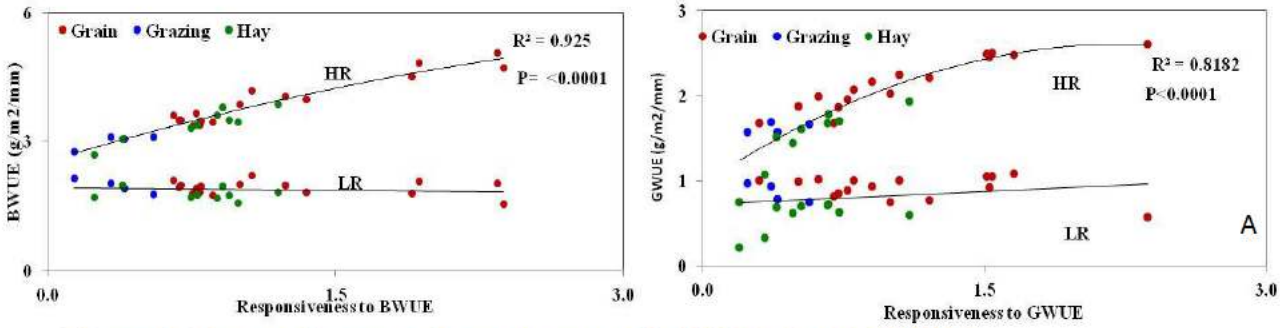
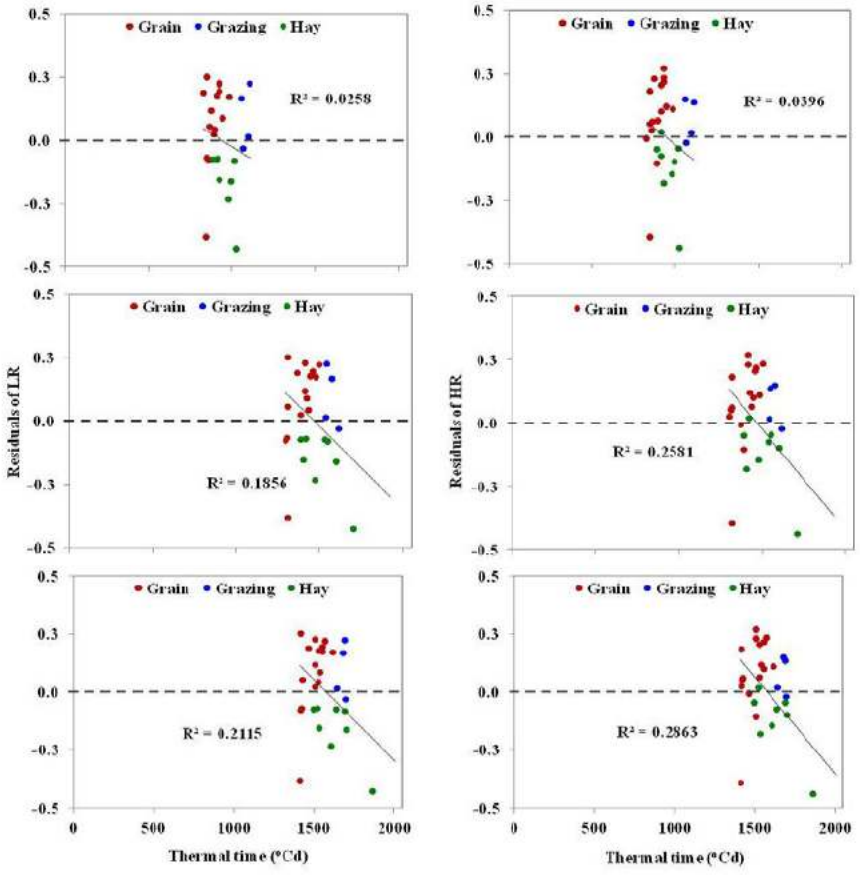


Figure 8. Responsiveness of genotypes to (A) BWUE & (B) GWUE



correlated in HR regions only (Figure 8) with no differences in biomass or grain WUE in LR regions. Grazing and hay types generally had lower BWUE and GWUE with lower responsiveness compared to grain types which were efficient in biomass or grain production under favourable conditions with positive contribution. Highest responsiveness (VR) for BWUE and GWUE was observed in 05014-22 (2.37 for both) followed by 05089-31 (2.34 for BUWE & 1.67 for GWUE) and Williams (1.92 for BWUE) and Bannister (1.54) for GWUE.

Figure 9. Relationship between residuals of GWUE vs responsiveness at LR & HR environments of genotypes growing degree days from sowing to SE, FL and HC.

Residual analysis for ET & BWUE showed no significance while residuals were significant for GWUE. The grain lines, 05014-22 & 05089-31 had high responsiveness but they negatively contributed (0.39 & 0.01 respectively) for GWUE while Mitika had the highest positive residuals (0.27) for GWUE followed by 05089-37 (0.23). GWUE also correlated with the growing degree days to FL and HC at both HR & LR showing differences in response to GWUE between early and late types. The results were similar to GY with late types become less efficient in using water for grain production (Figure 9).

BWUE and GWUE were not significant in LR. However, in HR there were significant differences between varieties/lines. The highest BWUE was observed in 05014-22 and

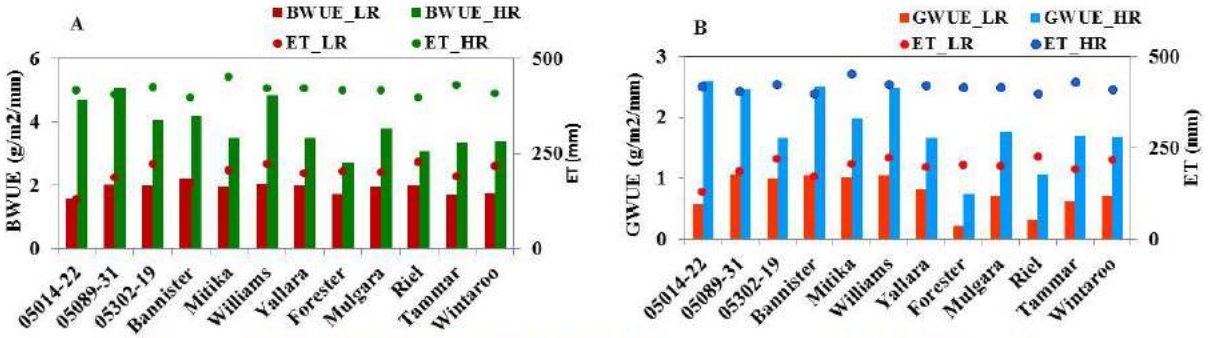


Figure 10 BWUE (A) & GWUE(B) of genotypes at LR & HR regions

Williams Figure 10A. The highest GWUE was 05014-22 followed by Bannister, Williams, and 05089-31 Figure 10B.

Figure 11A shows the grain water use efficiency (GWUE) and Figure 11B shows the biomass water use efficiency (BWUE) of the varieties/lines and ET at four site/years. There is little variation for ET by variety within an environment, but the environments cluster separately, indicating the significance of the environment.

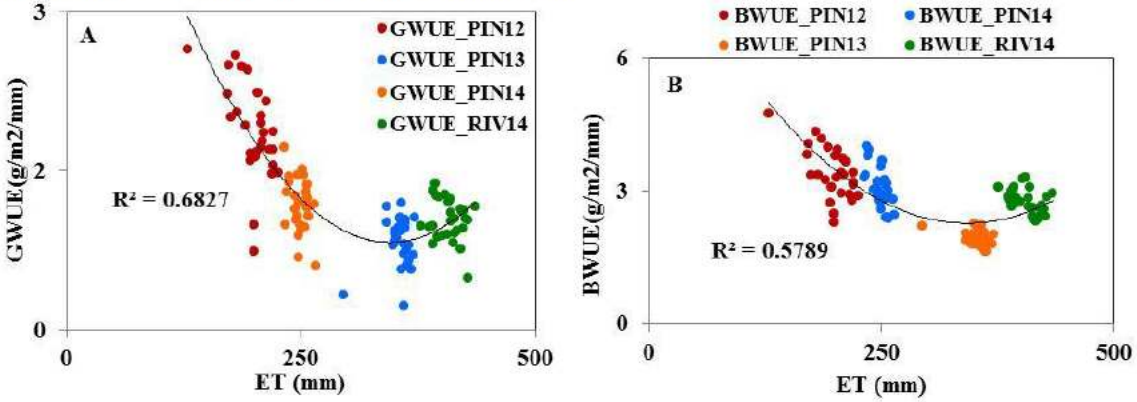


Figure 11. ET response of genotypes to environment vs GWUE (A) & BWUE (B)

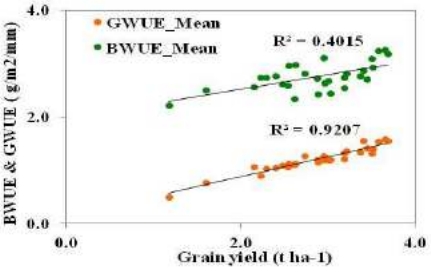


Figure 12. Correlation between GY & BWUE & GWUE

The mean BWUE & GWUE of the genotypes correlated with the mean GY of the genotypes with GWUE having stronger correlation (Figure 12).

Table 13. Wild crosses produced for study. The highlighted entries were promoted to F4

Entry	Name	Pedigree	Comment
1	11054WO	MITIKA/ IBERIAN-1174	
2	11176WO	04192-2/ IBERIAN-3076	
3	11178WO	YALLARA/ IBERIAN-3011	
4	11179WO	MITIKA/CC7209	
5	11180WO	MITIKA/IBERIAN-32	
6	11181WO	01164-35/CC7216	
7	11184WO	04203-40/IBERIAN-3151	
8	11185WO	YALLARA/ IBERIAN-321	
9	11186WO	03021-42/IBERIAN-3096	
10	11188WO	03021-42/CC7205	
11	11189WO	04136-31/IBERIAN-3019	
12	11192WO	04192-2/IBERIAN-24	
13	11206WO	WAOAT2354-SEL/IBERIAN-3076	
14	11209WO	04200-51/IBERIAN-3037	
15	11211WO	01164-35/IBERIAN-2156	
16	11212WO	WAOAT2332-SEL/IBERIAN-658	
17	11213WO	04203-18/IBERIAN-41	
18	11221WO	04136-31/CC7207	
19	11222WO	04290-3/IBERIAN-3053	
20	11223WO	03122-3/IBERIAN-1427	
21	11247WO	03014-1/IBERIAN-605	
22	11257WO	03122-3/CC7212	Too late for 2012 trial
23	11258WO	04290-3/CC7204	No seed
24	11259WO	FL03007-L1/IBERIAN-3282	
25	11260WO	MN06213-IBERIAN-30	Too late for 1012 trial
26	1126WO	ND040196-CC7208	