Australian Government

Australian Centre for International Agricultural Research

Final report

Project	**Indo-Australian project on root and establishment traits for greater water use efficiency in wheat- Phase 2**
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Contents

1 Acknowledgments

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2 Executive summary

Improving water use efficiency or water productivity (the ratio of yield to water use) of all crops is one of the most urgent global needs as water scarcity for agricultural use increases. In this Project we examined practical ways to genetically improve the water productivity of wheat in India and Australia.

Results from this Project (Phase 2) recommend two complementary practices to improve water productivity and yield. The first is for breeders to select lines with deeper roots that can access more water at depth so as to increase grain yield. Two novel, high throughput, selection criteria are recommended. Selecting for deeper roots has previously been elusive in breeding programs. The second practice is to sow earlier as this results in substantial yield gains due to both improved water use efficiency and deeper roots. This practice will further benefit from incorporating additional features which are easily selectable in breeding programs.

1. Deeper roots.
    - (i) We demonstrated that relatively simple, non-destructive, high throughput novel measurements can be used to select for deeper roots in segregating breeding populations. The measurements were canopy temperature and NDVI which we found to be above-ground proxies for deep roots. Both can now be routinely measured using land or aerial (ie unmanned aerial vehicles) devices. This has important implications for the breeding of new cultivars with deeper roots - a very difficult trait to select.
    - (ii) We demonstrated that proxies for deep roots that can be selected in controlled environments is not effective.

2. Crop establishment and earlier planting
    - (i) Earlier planting results in greater biomass and yield. This is because the crop duration is longer and so more light is intercepted for photosynthesis, water use efficiency is higher because there is more growth in the winter when water use efficiency is higher and the longer crop duration results in more root growth leading to more water and nutrient uptake. This is now possible with some current varieties.
    - (ii) To decrease the risk of poor crop establishment with earlier planting new dwarfing genes are recommended as these have longer coleoptiles and emerge better from warm soils than wheats with the Green Revolution dwarfing genes. Molecular markers are available for these new dwarfing genes.
    - (iii) It is also likely that different flowering alleles will be important so as to extend the duration of crop growth. These are known and they are important for increased diversity in breeding programs.

Both of these approaches have the potential to significantly improve wheat yields when water is limited. Developing wheat lines with deeper roots could have a long lead time whereas earlier planting could be implemented immediately although fine tuning with dwarfing genes and longer duration will take longer.

This Project was part of the India-Australia Program on Marker Assisted Wheat Breeding (IAP-MAWB). An objective of this Project was to develop molecular markers that could be implemented in breeding. This was not followed through in the ‘Deeper roots’ component of the Project as the root traits were too complex and phenotyping above-ground proxies was far more effective. Nevertheless, in the project component related to earlier sowing, molecular markers for dwarfing genes with long coleoptiles were made available by CSIRO. In addition, markers for key alleles that can extend the duration of growth and that could be important for earlier sowing are also available.

3 Background

India's population is estimated at 1.3 billion people, and around 2030, will take over that of China to become the world's most populous country. Wheat is India's second most important crop after rice, and is grown entirely on the Indian continent, satisfying current demand. However wheat productivity gain in India is slowing, like many areas around the world, and this century India faces the challenge of needing to increase wheat production to meet population growth, while irrigation water and land for agriculture declines due to urban expansion and industrialisation. India has a priority to develop new wheats with higher yields and higher water use efficiency faster than is currently being achieved with traditional "yield" breeding, to avoid food price increases, political instability, declining levels of education, and a slowing in economic growth.

This project (Phase 1 and Phase 2) meets India's research and development priority to accelerate wheat improvement, by selecting and incorporating beneficial yield and water use efficiency traits using phenotyping and marker assisted selection technologies. These technologies are expected to enrich for beneficial characteristics faster than selection for yield alone. The most beneficial characteristics in this project are (1) longer coleoptiles to allow farmers to plant deeper into soil, thus minimising effects of drought and crop loss at establishment and (2) deeper root systems to capture deep water often missed by the crop and very valuable to yield, especially during end of season droughts and environments that rely on rainfall. These characteristics were identified at a joint Workshop in 2007 in Delhi among Australian and Indian researchers. The potential value from these traits is high; for example proof of concept field experiments in Australia showed that small increases in root depth that give an extra 10 mm of water around grain development can result in an extra 0.5 tonnes of yield; 25% of the 2 tonne per ha average of the rainfed, central areas of India. This is large compared to the current rate of increase of 1% per annum in India and globally. The wheats will likely have a benefit in conservation farming systems of the future because they help emergence from retained plant matter in soils and help acquire deep irrigation water which is more efficient than surface water that rapidly evaporates.

This project meets two priorities for Australia: to increase its wheat productivity, and to increase the economic growth and stability of India, a major partner for trade.

Wheat is Australia's most valuable export crop and Australia has a research and development priority to increase yields to increase domestic economic growth, and to maintain rural regions. Further Australian farmers do not irrigate wheat, and almost all the wheat is rainfed. Australia also has a research and development priority to develop wheats with high water use efficiency. CSIRO and research agencies in Australia have a long history in development of wheats with greater water use efficiency, and in identifying characteristics of the wheat plant to specifically breed for, to increase its water use efficiency. This project has given Australia access to a new wheat germplasm pool from India, that has been selected over many years in regions of India that rely on deep water for yield. Phase 1 showed that these Indian wheats have deeper root systems than currently available in Australia cultivars, and in Phase 2, the Indian wheats and selection technologies are now available to Australian breeders to increase the yields and water use efficiency of wheat in Australia.

As part of the proof of concept activities, we will evaluate new non-destructive field phenotyping technologies developed by CSIRO that are expected to become routine tools in breeding in the future.

The project will benefit both India and Australia. By providing breeders in both countries with deep rooted germplasm and rapid, reliable selection methods, including molecular markers, this project facilitates the provision of new higher yielding and more water-use efficient germplasm to farmers in drier regions of both Australian and India.

Finally, we wished to build scientific capability in difficult and neglected areas of research. Specifically we have focused on root systems and below-ground activities which are not seen and therefore not easily studied. The Project is also linked with ACIAR John Allwright PhD Fellow Ms. Ritika Choudhary, formerly with the University of Sydney and CSIRO, but now based at Karnal, to evaluate the potential for earlier sowing of wheat seed to allow breeders to use new varieties developed in this project to be sown earlier in India and Australia, hence increasing water use efficiency and avoiding drought at the end of the season. We assembled a highly committed group of junior Indian and Australia scientists throughout both phases of the Project; it is notable that most were female.

4 Objectives

Phase 2

1: Select wheat genotypes to be donors of deep root traits in breeding

2: Develop phenotypic screens so as to develop for molecular markers and elite lines with deep roots for breeders

3: Develop molecular markers for deep roots

4: Develop wheat genotypes with deep roots and elite adaptations

5: Provide field proof that genotypes with deep roots and good establishment traits increase water uptake and yield

6: Build capability root research in India and Australia

5 Methodology

Success in improving physiological traits such as deeper growing root systems and improved crop establishment are first dependent on having appropriate germplasm and second in having a fast and efficient screening technique.

CSIRO has had a long term interest in identifying novel wheat germplasm for improved root growth and improved crop establishment. It has been a world leader in identifying the problem of poor establishment associated with current breeding germplasm containing the dwarfing genes Rht1 and Rht2 which universally has short coleoptiles. CSIRO has identified novel germplasm containing genes which retain the desired height associated with Rht1 and Rht2 but have long coleoptiles. These genes are designated Rht4, Rht5, Rht8, Rht12, Rht13, Rht14 and Rht18. As part of this project wheat lines containing these genes were sent to all collaborators in India and were used extensively in Australia. Individual genes have not been associated with deeper roots. Nevertheless, germplasm studied closely at CSIRO has been found to possess traits associated with deeper roots. For example, high early vigour germplasm has been developed at CSIRO that also has vigorous root systems. In addition germplasm with the tin gene, which inhibits tiller development, has also been associated with deeper roots. CSIRO has also identified other germplasm which could be important for deep root growth. For example, genes that control flowering have also been associated with deeper roots because there is an extended period of time for deep root growth, synthetic germplasm originating from CIMMYT have been implicated in deeper root growth. It is also possible that wheat cultivars bred in India and Australia in regions where crops are reliant on stored soil water may also have been inadvertently bred with deeper roots.

Overall about 500 unique wheat lines were assembled in Australia for this Project and sent to India. These formed the basis of the germplasm screening, breeding and evaluation in India and Australia.

The second requirement of a successful selection program for deep roots and long coleoptiles is a reliable way to screen for these traits which is fast and repeatable. CSIRO has developed a number of effective screening tools for long coleoptiles that are very reliable and these have been shared with Indian collaborators. However, screening for deep roots has been very elusive. This is for a number of reasons. Firstly, you cannot see them. Secondly, roots grow in the field non-randomly; they cannot penetrate hard soils and so will find the paths of least resistance where there is moisture. Thus, they tend to ‘search’ for cracks and pores in the soil. In this project we have attempted many screens for fast growing roots that are quick and reliable and are related to root growth in the field. In controlled environments we developed screens in moist paper towels which can identify roots which grow fast and which are angled vertically. We have developed ways of evaluating whether wheat genotypes grow roots which are more vertically inclined in the soil and ways of measuring root mass in 50 cm tubes. Although these methods could be used to screen for roots they are artificial and we cannot identify relationships with field grown roots.

In this Project we have devoted substantial resources into developing fast and efficient field screens that can be used to identify wheat lines in the field that have deep roots. The most important one has been to develop a field coring techniques that is fast and repeatable. Accordingly we developed a tractor mounted system that can hydraulically core and extract roots to 2m deep in the soil. This has become the gold standard internationally. After extracting the core it is laid out horizontally and by breaking the soil core every 10cm we can see how deep the roots are and how frequent the roots are. Thus, we do not have to wash the roots. We have tested this in a number of different field layouts. We have grown standard field plots which are typically 5m long and 2 m wide and also hill plots where seeds of each wheat line are planted in a clump 50cm from the next genotype. We have also experimented with growing a common wheat line as a border to every hill so that there is equal competitive effects. The advantage of this method is that hundreds of lines can be sown in a small space and we can eliminate soil variability often associated with large plots. We then take a soil core from each hill plot or from each larger plot.

In addition to soil coring to monitor actual root depth we have examined proxies for deep roots. The thinking behind this is that any line with deeper roots than its neighbour which is growing on a soil where there is water at depth but declining water in the topsoil should stay green for longer and be cooler. We have thus, devised techniques to measure canopy temperature on hill plots and plots using high throughput aerial techniques as well as objective measurements of canopy greenness.

Although coring was successful in identifying extreme lines with deep roots the variability encountered due to subtle differences in soils that altered root growth it was not possible to use this in screening or in molecular marker identification. Accordingly we decided to test measurements that could be made quickly and easily on the above-ground plant that may reflect the depth of the root system. We assumed that any line with a deep root system would remain greener for a longer period than lines with a shallow root system when the crop is growing on sub soil moisture. We also assumed that the leaves of these plants would keep their stomata more open and for longer. Lines with more open stomata have cooler leaves. Useful advances have been made in recent years to measure both canopy temperature and canopy greenness using non-destructive techniques. NDVI (Normalised Difference Vegetation Index) is an index which measures greenness. There are low-cost hand held devices (eg Green Seeker) which can also be mounted to mobile farm equipment that can measure NDVI and cheap hand held infra-red thermometers that measure leaf temperature. However, relatively low-cost thermal imagery cameras can also be used that take infrared photographs that can be converted to temperature which can also be mounted on drones, helicopters or aeroplanes. These can be used to take instantaneous measurements on hundreds of field plots at a time or tens of thousands of hill plots.

6 Milestone report for Phase 2

6.1 Objective 1: To identify wheat genotypes with deeper, faster-growing root systems that access more soil water and result in greater yield

No.	Activity	Outputs/ milestones	Completion date	Comments
1.1	Team members select wheats with deep root traits	Wheats with deep roots selected	August 2013	Achieved. Manuscript published (Rich et al., 2016)
1.2	Parents with deep root traits made known to breeder, and seed supplied.	Information on wheat lines carrying deep root traits	February 2014	Achieved. Manuscript published (Rich et al., 2016)

6.2 Objective 2: Select phenotypic screens to develop molecular markers and elite varieties with deep roots for breeders (PC and A)

No.	Activity	Outputs/ milestones	Completion date	Comments
2.1	Team members identify fast and repeatable phenotypic screens for depth and distribution	M3: Screen selected lines (Obj.1.1) for root developmental traits. (A)	October 2014	Achieved. Controlled environment screens were not related to rooting depth of mature wheat plants in the field. Proxies related to canopy temperature and maintenance of green leaf area in the field were found to predict rooting depth. Publications close to submission (Rich et al., 2019; Xiaoxi et al., 2019).
2.2	Researchers and breeders in India and Australia provided with these phenotypic screens.	M4: Present methods to breeders via meetings and publications. (A, PC)	February 2015	Achieved. Publications close to submission (Rich et al., 2019; Xiaoxi et al., 2019)

6.3 Objective 3: To develop molecular markers for deep roots

No.	Activity	Outputs/ milestones	Completion date	Comments
3.1	Indian and Australian team members collectively select wheats with shallow root traits that are widely contrasting from the deep donors, to be shallow root donor parents	M5: Shallow rooted wheats selected. (A, PC)	August 2013	Achieved. See Rich et al. (2016)
3.2	Develop two mapped doubled haploid (DH) populations and map an existing (recombinant inbred line) RIL population (outsourced).	M6: Produce two mapped double haploid populations. Map existing RIL population from phase 1. (A, outsourced)	July 2015	Populations have been developed. Mapping was not completed because of on-going concerns over the most suitable phenotypic screen. (For mapping you must have a suitable phenotypic screen.)
3.3	Phenotype DH and RIL populations in India and Australia for deep roots.	M7: Phenotype developed populations for relevant root traits using CE screened developed in (Obj 2.1). (A, PC) M8: Phenotype developed populations for relevant root traits using field coring methods developed during phase 1. (A, PC)	Jan 2016 (controlled environment phenotyping) Dec 2016 (field phenotyping	On-going. Delays in the field due to weather events preventing satisfactory field experiments. The Project was extended at no cost so that a field season could be completed satisfactorily in Australia. Controlled environment phenotyping needed to match field results before the task of population phenotyping was undertaken. Research continued to develop controlled environment screens. See Rich et al. (2019). Field coring was unsatisfactory due to soil heterogeneity. New field phenotyping techniques were successfully implemented in the 2017 season using CSIRO Phenomobile and air-borne canopy temperature techniques. See Xiao et al. (2019)

3.4	Identify quantitative trait loci (QTL) associated with root phenotypes.	M9: Identify genomic regions associated with relevant root traits. (A)	Jan 2017	QTL were not identified as we established that direct field phenotyping for deep roots was not feasible.
3.5	Identify marker(s) for deep root traits and validate them in elite wheats developed by back cross breeding (Obj. 4).	M10: Markers for relevant root traits. (A) M11: Markers validated in different genetic background. (A)	July 2017 July 2017	Backcross populations were developed to the BC1F2 stage. They were not taken beyond this because a satisfactory and repeatable phenotype has not been identified for deep roots to enable backcrossing to be undertaken. For the same reasons validation of markers in different genetic backgrounds was not possible.
3.6	Provide Indian and Australian breeders with markers for deep root traits.	M12: Present methods to breeders via meetings and publications. (A, PC)	July 2017	Not achieved. Deep roots is a very elusive trait that is controlled by many genes each having a minor effect. For this reason the QTL are unlikely to be implemented in breeding.

6.4 Objective 4: Develop wheat genotypes with deep roots and elite adaptations

No.	Activity	Outputs/ milestones	Completion date	Comment
4.1	Identify elite varieties with adaptations to climatic zones	M13: Elite adapted wheats selected. (A, PC)	August 2013	Completed
4.2	Teams cross root donors with adapted varieties in a back cross breeding program that enriches for deep roots while retaining adaptations	M14: Adapted deep rooted wheats. (A, PC)	July 2016	Completed. However, backcrossing beyond BC1F2 has not been possible due to not having a reliable phenotypic screen.

4.3	Elite wheats with deep roots and adaptations to different climate provided to breeders.	M15: Adapted deep rooted wheats made available to breeders. (A, PC)	July 2017	Achieved in early 2018 as weather events preventing field phenotyping in 2016. Note that Project was extended into 2018 at no cost to complete the field phenotyping (Xiaoxi et al. (2019).

6.5 Objective 5: Provide field proof that genotypes with deep roots and good establishment traits increase water uptake and yield

No.	Activity	Outputs/ milestones	Completion date	Comments
5.1	Demonstration in the field that long coleoptile, low tillering and soil shading genotypes access more water than current varieties	M16: Field experiments using soil water and water use efficiency measurements to show water use of long coleoptile, low tillering and soil shading genotypes. (PC)	July 2016	Achieved in part. Long coleoptile and soil shading genotypes have higher water-use efficiency. Water use measurements in the field are very complicated and have not been accomplished. These measurements were also conducted with plants in 2m long tubes grown outside.
5.2	Demonstration in the field that deep rooted genotypes assess more deep water	M17: Field experiments using soil water and water use efficiency measurements to show water use deep rooted genotypes (A, PC)	July 2017	Achieved. Note that weather events prevented field experiments in 2016 and Project was been was extended into 2018. Data being reported in Xiaoxi et al. (2019)

PC = partner country, A = Australia

6.6 Objective 6: Build capability root research in India and Eastern regions

No.	Activity	Outputs/ milestones	Due date of output/ milestone	Comments
6.1	Host one Indian junior scientist for 12 to 18 months, plus a possible second Bangladeshi junior scientist at CSIRO.	M18: Host Indian junior scientist and possibly a Bangladeshi scientist	July 2017	This was not initiated nor was hosting of Bangladeshi junior scientist
6.2	Facilitate development of junior scientists and PhD student associated with the project through visits, meetings, leadership opportunities, data analysis and publications.	M19: Junior scientists and PhD student participate in visits, complete and analyse research and co-author conference presentations and publications.	July 2017	Collaboration with junior scientists undertaken including hosting of junior scientist from Indore during an International root meeting. Close supervision of PhD student involved in field, controlled environment and molecular marker activities
6.3	Collaborate with John Allwright ACIAR-funded PhD student Ritika Chowdhary	M20: Student participates in project meetings, has germplasm, expands network	July 2017	Scholarship extended to 30 Dec 2018 due to maternity leave.
6.4	Engage a new partner from Eastern India into the team	M21: New partner participates in project meetings and helps achieve project milestones in Eastern India.	July 2017	This was not initiated

7 Key results and discussion

7.1 Deep Roots

This has been a pioneering project into deep roots for water extraction and it has established itself as a world class project.

Development and evaluation of screening methods

An extremely diverse and large collection of germplasm varying in many traits likely to influence deep roots was evaluated in controlled environments as well as managed field environments. Despite many attempts to explore root systems in controlled environments we were not successful in developing a technique that replicated the result in the field. We are in the process of submitting a manuscript to a major international journal reporting this (Rich et al., 2019). During the course of the Project several methods were proposed in the international literature as being successful in being able to identify lines with deep roots. We were unable to replicate these findings.

One of the reasons for the success of the Project was that we were able to develop a tractor mounted field coring device that was able to core about 200 plots to 2 m deep in a single day. This is believed to be the highest throughput for field coring of its kind in the world and we reported this (Wasson et al., 2014). However, even this proved to be unsuitable for effectively screening large populations of lines for deep roots. The reason being that field soils are so variable that very extensive replication is required due to small local variation in soil properties. It also suffered from being very labour intensive requiring 4 operators.

To minimise the variation in soil physical properties we devised a novel planting arrangement for screening large numbers of lines. We grew hill plots, where clumps of seeds of the same genotype were grown on a 0.5 m grid. In a 100 m2 area it was possible to grow 375 hill plots but only 16 standard breeder plots. This resulted in a minimisation of soil variability in the hill plots. This also has substantial benefits where space is a premium such as in India. A further modification of the hill plots to eliminate hill plot to hill plot variability was to sow a winter wheat check to border all hill plots. The winter wheat remains largely vegetative as a border whereas the test hill plot is a spring wheat and grows to maturity. With this arrangement it is possible to grow 180 hill plots in a 100 m2 area. We reported this in Wasson et al., 2014).

Although coring was successful in identifying extreme lines with deep roots the variability encountered due to subtle differences in soils that altered root growth it was not possible to use this in screening or in molecular marker identification. Nevertheless we did identify parent lines that we used to develop doubled haploid populations. Lines that were deepest rooting in the field, where there was water at depth and a drying top soil, were two older Indian wheat C306 and H1500 (See Rich et al., 2019).

Based on the above findings we decided to test measurements that could be made quickly and easily on the above-ground plots that may reflect the depth of the root system. We assumed that any line with a deep root system would remain greener for a longer period than lines with a shallow root system when the crop is growing on sub-soil moisture. We also assumed that the leaves of these plants would keep their stomata more open and for longer. Lines with more open stomata have cooler leaves. Great advances have been made in recent years to measure both canopy temperature and canopy greenness using non-destructive techniques. NDVI (Normalised Difference Vegetation Index) is an index which measures greenness. There are low-cost hand held devices (eg Green Seeker) which can also be mounted to mobile farm equipment that can measure NDVI and cheap hand held infra-red thermometers that measure leaf temperature. However, relatively low-cost thermal imagery cameras can also be used that take infrared photographs that can be converted to temperature which can also be mounted on drones, helicopters or aeroplanes. These can be used to take instantaneous measurements on hundreds of field plots at a time or tens of thousands of hill plots.

We tested these ideas on three wheat populations. One of which was developed in this project and another two were developed by CSIRO. We measured 1800 plots multiple times for NDVI and canopy temperature hence demonstrating the high throughput nature of these techniques and their suitability in breeding. To determine the relationship of these proxies with rooting depth we selected about 50 extremes for canopy temperature and NDVI in the 3 populations. In total we cored almost 300 plots and we took 4 soil cores from each plot on which we determined the depth of the deepest roots. In summary canopy temperature and NDVI successfully predicted which lines had the deepest roots in all populations. In two populations the grain yield of the lines with the deepest roots were 19 and 7% higher.

Management practices resulting in deeper roots

A second important finding we made during the course of this Project is that rooting depth depends on sowing date. The longer the duration of growth to flowering then the deeper are the roots. What this means is that if sowing is earlier and lines flower at the same optimal time then the roots will be longer and more able to take up water and nutrients for higher yield. This is because there is more time to grow both deeper and more branched root system. This has been tested with sowing date studies in both India and Australia. Other advantages associated with the earlier sowing is that as well as more time to use water and nutrients for crop yield the use of the water by the crop is more efficient. That is more biomass is produced per mm of water used.

To achieve the benefits from early sowing it is likely that new varieties will be required that will flower at the optimal time and not too early. This should not be difficult to manage in practice as flowering time is highly heritable and easily selected for in breeding programs. Furthermore, there are very good molecular markers that can be used to assist in selection.

The major disadvantage with earlier sowing is that the soils are usually hotter and drier and this can result in poor crop emergence.

Summary

A. We have provided strong evidence that relatively simple measurements on above-ground proxies that were predicted to reflect root system function can be used to select for deeper roots and for greater grain yield. This has important implications for the breeding of a very difficult trait in major crops. It will be important for regions of India and Australia (and indeed other wheat producing regions) where water for crop production is limited (Xiaoxi et al. 2019).
B. We have demonstrated that proxies for deep roots that can be selected in controlled environments is not effective (Rich et al. 2019
C. Provided there is no change in flowering time then earlier sowing is a simple practice that will ensure deeper roots and access to more soil water. It also results in a higher water-use efficiency and usually higher yields (Richards et al., 2014).

7.2 Crop Establishment

The ‘Green Revolution’ dwarfing genes in wheat, referred to as Rht-B1b and Rht-D1b, have had a dramatic impact globally. They are ideally suited for high production systems where irrigation, nitrogen fertiliser and good seed beds are available. Unfortunately they are not suitable for production systems where crops are sown into unfavourable seed beds which may be dry and hot. The reason for this is that the Green Revolution dwarfing genes result in very short coleoptiles, ie the cylinder that protects the first leaf as it emerges from the seed to the soil surface. If the seed is sown deep then poor crop establishment and low grain yield results. The coleoptile is also susceptible to warm temperatures as an increase in soil temperature shortens the coleoptile again resulting in poor establishment and sometimes in crop failure.

CSIRO has been a leader in the identification of new dwarfing genes that have the same yield as the Green Revolution dwarfing genes but they have long coleoptiles. This makes them far more suitable than the current dwarfing genes when crop establishment occurs under sub-optimal conditions. CSIRO germplasm containing the most important dwarfing genes were introduced into India at the commencement of this Project. Molecular markers that can be used to successfully identify the dwarfing genes were also shared with all collaborators in India. This germplasm has been used in multiple experiments and in breeding.

A major finding in the project is the greater biomass and yield that occurs with earlier planting. This is because early planting can result in a deeper and more extensive root system resulting in greater uptake of water and nutrients for higher yield. It also results in a higher water-use efficiency. However, if this is practiced then new varieties must be developed with new dwarfing genes that enable better crop establishment.

Summary

D. Earlier planting results in greater biomass and yield and more efficient use of water and nutrients. Earlier planting can be implemented immediately. However, it will benefit greatly from the introduction of new dwarfing genes to enable better establishment (Richards et al., 2014). It may also require a genetic adjustment to flowering time. Molecular markers are available to assist with this breeding and selection.

8 Impacts

8.1 Scientific impacts – now and in 5 years

The findings from this research, if adopted, have the potential to increase wheat production substantially.

1. Earlier sowing, provided great care is taken with good seed bed preparation, could raise wheat production in areas where it could be practiced by at least 10% for no additional costs. It would result in additional benefits of improved water use efficiency and increase nutrient use efficiency. It may require the use of later flowering cultivars (which are likely to be already available).
   This finding could be implemented immediately.

2. Introduction of new dwarfing genes and introduction of alleles to enable later flowering would result in substantial further benefits. New dwarfing genes would make earlier planting much more robust and protect against poor crop establishment and crop failure. Later flowering would improve crop adaptation and ensure the benefits of longer crop duration made possible by the earlier planting are translated into improved yields and efficiencies.
   Breeding could be commenced immediately to introduce new dwarfing genes and adjustment of flowering time. Germplasm and markers have been provided to all collaborators.

3. This is the first ever demonstration that deep roots can be selected easily in large breeding populations using canopy temperature and/or NDVI. It is also the first time that substantial yield benefits (up to 19% here) have been shown in large populations. Selection using NDVI and/or canopy temperature can now be readily implemented in breeding programs. NDVI can be used to screen about 400 plots per hour using a relatively inexpensive 'Green Seeker' whereas canopy temperature can be measured using a UAV and a thermal infrared camera. Thousands of plots can be measured in a single fly-over.
   Selection using NDVI and canopy temperature could be implemented immediately in breeding programs.

8.2 Capacity impacts – now and in 5 years

Drs Sarah Rich, Cathrine Ingvordsen and Xiaoxi Li were post-doctoral fellows on this Project at different times in Australia. After a period of maternity leave Sarah Rich was appointed as a PDF with CSIRO Agriculture and Food in Perth. Cathrine Ingvordsen was appointed as a PDF in pre-breeding at CSIRO Agriculture and Food in Canberra. Xiaoxi Li was appointed to a PDF position at CSIRO Agriculture and Food in Canberra.

The development of a world class soil coring capability to measure root system depth in the field was developed by Dr Anton Wasson in Phase 1 of this Project. This was used in Phase 2 of the project. It remains the gold standard globally.

Phenotyping large populations using remote sensing was used extensively in the final stage of this project. This was made possible by the CSIRO High Resolution Plant Phenomics Centre. This Project provided one of the first demonstrations of the value of high throughput phenotyping for hard to measure traits and its application in plant breeding. Mick Weiss, a technical officer on the project, was responsible for the collection of much of this data.

Ms Ritika Chowdhary was a John Allright Fellow and a PhD student associated with the Project. Ritika worked on emergence of wheat for early sowing. She took maternity leave from November 2016 and later returned to complete her PhD. Ritika is currently in India writing up her research at Karnal.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

Adoption of early sowing of wheat in appropriate regions of India is expected to have an immediate economic impact. This will come from early sown crops having deeper roots and therefore access to more water, a higher water use efficiency and a more effective use of fertiliser. Further breeding is likely to improve yields more and remove some of the risk associated with earlier sowing.

8.3.2 Social impacts

Not possible to assess.

8.3.3 Environmental impacts

Earlier sowing and genetically improved root systems will result in higher water use and nutrient use efficiency and thereby more sustainable agricultural systems.

8.4 Communication and dissemination activities

Peer reviewed scientific publications from this Phase 2 Project are the main form of communication.

These are as follows:

Xiaoxi Li, Cathrine Ingvordsen, Michael Weiss, Greg J. Rebetzke, Anthony G. Condon, Richard A. James, Richard A. Richards. (2019). Deeper roots and greater yields associated with cooler canopies and higher NDVI after anthesis in three wheat populations grown on stored soil water. Journal of Experimental Botany (in final draft form).

Rich, S.M., Wasson, A.P., Richards, R.A., Katore, T., Prashar, R., Chowdhary, R., Saxena, D.C., Mamrutha, H.M., Zwart, A., Misra, S.C., Prasad, S.V.S., Chatrath, R., Christopher, J., Watt, M., (2016). Wheats developed for high yield on stored soil moisture have deep vigorous root systems. Functional Plant Biology 43, 173-188.

Rich, S.M., Christopher, J., Richards, R.A. and Watt, M. (2019). Root traits at the vegetative stage correlate poorly with genetic variation in mature root traits in the field. (in final draft form).

Richards, R.A., Hunt, J.R., Kirkegaard, J.A., Passioura, J.B., (2014). Yield improvement and adaptation of wheat to water-limited environments in Australia-a case study. Crop & Pasture Science 65, 676-689.

Wasson, A.P., Rebetzke, G.J., Kirkegaard, J.A., Christopher, J., Richards, R.A., Watt, M., (2014). Soil coring at multiple field environments can directly quantify variation in deep root traits to select wheat genotypes for breeding. Journal of Experimental Botany 65, 6231-6249.

Wasson, A., Leanne Bischof, Alec Zwart, Michelle Watt.(2016). A portable fluorescence spectroscopy imaging system for automated root phenotyping in soil cores in the field. Journal of Experimental Botany, 67(4):1033-1043.

Conference presentations and posters have also been made. These are as follows:

Posters:

International Wheat Conference, Sydney 20-25 September 2015

Identification of new genetic variation for high temperature tolerance in wheat seedlings. Ritika Chowdhary, Sarah M Rich, Richard A Richards, Greg Rebetzke, Cathrine H. Ingvordsen, Ravish Chatrath, Richard Trethowan, Michelle Watt.

International Symposium, International Society of Root Research, Canberra 6-9 October 2015

Is root growth in wheat compromised during stem elongation and grain filling?

Cathrine H Ingvordsen, Sarah M Rich, Michael Weiss, Anton P Wasson, Richard A Richards, Michelle Watt.

Root depth and yield of wheat in three Indian environments. Ritika Chowdhary, Renu Parashar, Trushna D Katore, Ravish Chatrath, SV Sai Prasad Satish C Misra, Mamrutha HM, Dinesh C Saxena, Omprakash Tuteja, J Bagwan, Anton Wasson, Richard Richards, Greg Rebetzke, Michelle Watt.

Oral presentations:

International Wheat Conference, Sydney September 20th-25th 2015. Deep-root phenotypes in wheat can be exploited in breeding to increase grain yield. Sarah M Rich, Anton Wasson, Cathrine H Ingvordsen, Ritika Chowdhary, Michael Weiss, Greg Rebetzke, Ravish Chatrath, Michelle Watt, Richard Richards.

9 Conclusions and recommendations

9.1 Conclusions

Deep Roots

We have gathered strong evidence to show that relatively simple measurements on above-ground proxies that were predicted to reflect root system function can be used to select for deeper roots and for greater grain yield. This has important implications for the breeding of a very difficult trait in major crops. It will be important for regions of India and Australia (and indeed other wheat producing regions) where water for crop production is limited.

We have demonstrated that proxies for deep roots that can be selected in controlled environments is not effective.

Provided there is no change in flowering time then earlier sowing is a simple practice that will ensure deeper roots and access to more soil water. It also results in a higher water-use efficiency.

Crop Establishment

Earlier planting results in greater biomass and yield and more efficient use of water and nutrients. However, it will benefit greatly from the introduction of new dwarfing genes to enable good establishment. It may also require a genetic adjustment to flowering time. Molecular markers are available to assist with this breeding and selection.

The practice of earlier planting will also result in a substantial improvement in both water use efficiency and nutrient use efficiency thereby leading to a reduction in on-farm costs and additional environmental benefits.

These conclusions have been derived from experiments with wheat. They are also likely to be applicable to other temperate crops.

9.2 Recommendations

1. That wheat growers with the option of earlier sowing implement this practice. It has many benefits. Root systems are deeper and extract more water and nutrients and use these very efficiently. This, plus the extra duration of light capture, results in substantial improvements in above-ground biomass and grain yield. There are caveats here in relation to seed beds at the time of seeding.
2. That wheat breeders extend the duration of flowering as part of their breeding practice. It is very important that diversity in flowering is available for growers who may want to adjust their management practices. In this it is important that the optimal time of flowering is achieved but that there is flexibility in planting time.
3. That wheat breeders introduce new dwarfing genes with long coleoptiles. These will reduce the risk of poor emergence that can be associated with earlier planting into soils that may be hotter. So far no penalties have been associated with these dwarfing genes.
4. That wheat breeders in regions where soil moisture may be available at depth in some years initiate selection for deeper roots using the proxy of NDVI or canopy temperature. Thousands of lines can be measured in a single day using this technique. The traits are also highly repeatable. These traits may also have benefits under favourable conditions. There is unlikely to be any yield trade-off with deeper roots as if there is no water at depth then the deeper rooting trait will not be expressed.

10 List of publications produced by project

10.1 Scientific publications in International journals

Xiaoxi Li, Cathrine Ingvordsen, Michael Weiss, Greg J. Rebetzke, Anthony G. Condon, Richard A. James, Richard A. Richards. (2019). Deeper roots and greater yields associated with cooler canopies and higher NDVI after anthesis in three wheat populations grown on stored soil water. Journal of Experimental Botany (in final draft form).

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10.2 Poster publications:

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Identification of new genetic variation for high temperature tolerance in wheat seedlings. Ritika Chowdhary, Sarah M Rich, Richard A Richards, Greg Rebetzke, Cathrine H. Ingvordsen, Ravish Chatrath, Richard Trethowan, Michelle Watt.

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