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Final report

Small research and development activity

project

Applying wheat quality markers in India

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1 Acknowledgments

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2 Executive summary

Biscuits are widely consumed in India, but elite biscuit quality cultivars are not available to Indian farmers or industry. Australia has elite biscuit cultivars and extensive knowledge of many of the genes required for elite biscuit quality. Specific alleles of both glutenin and puroindoline genes are required and these can be identified by diagnostic molecular markers. This provides the opportunity to establish a breeding program with the immediate objective of developing biscuit cultivars, but also, in the longer term, as a model for integrating cross prediction technologies based on molecular markers into Indian wheat breeding.

Five elite Australian biscuit cultivars, Barham, Yenda, EGA Jitarning, EGA 2248 and Orion were identified and exported to India. These were characterised for 24 genes, including those known to be required for biscuit quality, adaptation and disease resistance. A further fourteen Australian cultivars, known to already be available in India, and 15 diverse Indian cultivars were also characterised for the same 24 genes. In collaboration with Prof Richard Trethowan, these characterisations were entered into the ICIS database which is being used by Indian wheat breeders. Importantly, this characterisation of 34 cultivars for 24 genes made a reference set that could be used to facilitate the use of molecular markers in Indian wheat breeding programs.

Protocols were written to assist with the use of these markers. Already, requests have been made through the Generation Challenge Program for these marker protocols to be distributed more widely than just to the participants of this SRA. We recommend that approval be given for their wider distribution.

Diagnostic markers for grain quality and adaptation were used to predict the outcome of crossing cultivars like Yenda with Indian cultivars adapted to north-western India. We concluded that marker-based selection among BC_1F_1 plants would greatly improve the frequency of desirable progeny from these crosses. This methodology was discussed with Dr G.P. Singh, who plans to continue with the development of biscuit cultivars in India. His colleague, Dr Mahendru Singh, has the equipment and skills required for the completion of this project, so long as it remains a priority for Indian wheat breeding. We therefore concluded that there were no grounds to continue this SRA with a larger project.

3 Introduction

Biscuits are widely consumed in India, but elite biscuit quality cultivars are not available to Indian farmers or industry. Australia has elite biscuit cultivars that are expected to perform in India at levels below current elite cultivars, but at levels beyond those of landraces and many other potential sources of genes needed to develop biscuit cultivars adapted to Indian conditions. Furthermore, from extensive research, a great deal is known about the genetic basis of biscuit quality in Australia. This provides the opportunity to use the establishment of a breeding program for biscuit cultivars to be used for the immediate purpose of developing biscuit cultivars, but also, in the longer term, as a model for integrating cross prediction technologies into Indian wheat breeding.

Activities in this SRA fell into three broad areas:

1. The identification and transfer to India of elite Australian biscuit wheat cultivars that could be used as parents in the breeding of biscuit cultivars adapted to Indian conditions.
2. The identification of a reference set of Indian and Australian cultivars that could subsequently be characterised with diagnostic markers for genes controlling grain quality, phenology, disease resistance and tolerance of abiotic stresses. These characterisations could then be used as controls for Indian molecular scientists associated with breeding programs to use in Indian wheat breeding programs, just as they are in Australian programs. They would also be valuable for future collaboration between India and Australia in molecular wheat breeding.
3. To work with Indian wheat breeders on useful strategies for using molecular markers for designing crosses for developing biscuit cultivars, but with the longer-term objective of the wider use of markers for all classes of wheat. This activity overlapped substantially with activities in CIM/2005/020 ‘Molecular marker technologies for faster wheat breeding in India’, led by Prof Richard Trethowan.

4 Activities

4.1 Identification of Australian biscuit wheat cultivars to send to India

After consultation with Dr Russell Eastwood, AGT, Dr Robyn McLean, InterGrain, and Dr Marie Appelbee, LongReach, all successful Australian breeders of biscuit cultivars, the following elite cultivars were identified:

- Barham
- Yenda
- EGA Jitarning
- EGA 2248
- Orion

As indicated from the pedigrees in ICIS, these cultivars are not closely related. All are suitable for the Australian Soft class, but, in addition, some of these cultivars carry genes for tolerance to abiotic stresses and resistance to diseases that should be useful to Indian wheat breeders beyond their value as donors of genes for biscuit quality. For example, Yenda was known to carry the *Bo1* gene for tolerance to high levels of boron and the *Rlnn1* gene for resistance to root lesion nematodes. These characteristics were considered in the selection of these cultivars.

Through correspondence with Dr Mahendru Singh, importation permits for India were obtained and pure seed of the cultivars was sent from the Australian Winter Cereals Collection to India on 24 September 2010, in time for the coming rabi season. Subsequently, Drs Cane and Eagles observed the cultivars being successfully grown at IARI in Delhi on 18 February 2011, with crosses already being made to selected Indian cultivars. Dr Eagles was pleasantly surprised to find that these Australian cultivars were flowering almost simultaneously with adapted Indian cultivars, which augurs well for their use in Indian wheat breeding.

4.2 Identification and characterisation of the reference set

At a meeting on 11 September, 2010, in Karnal a set of 15 Indian cultivars were identified for use in a reference set. This meeting was chaired by Dr S.S. Singh. These cultivars were selected to represent diversity of Indian germplasm and to be complementary to Australian cultivars already in India. They included both modern and older cultivars. In addition, 19 diverse Australian cultivars were identified. Seed of the Australian cultivars had to already be in India, and were also chosen for their known diversity. The five biscuit cultivars were included.

At this meeting, genes with diagnostic markers of interest to both Indian and Australian wheat breeders were identified.

The final set included the following genes affecting grain quality:

Glu-A1, Glu-B1, Glu-D1, Glu-A3, Glu-B3, Glu-D3, Pina-D1, Pinb-D1, Srp5B, Spa-A1, Spa-B1, Spa-D1

The final set included the following genes affecting plant phenology, morphology and disease resistance:

Ppd-D1, Vrn-A1, Vrn-B1, Vrn-D1, Rht-B1, Rht-D1, VPM-1, *Sr24/Lr24, Lr34/Yr18, Sr2, Tsn1, Sr26*

Seed of the selected Indian cultivars was obtained in Australia from Prof. Trethowan. In the laboratory of Dr Karen Cane, the full reference was characterised for the above genes. These results were sent to India in detailed spreadsheets, with copies also sent to Dr Paul Fox at ACIAR. Our Indian colleagues characterised genes and cultivars of interest, for example Dr Sewa Ram at DWR in Karnal was especially interested in *Spa* genes, as potentially involved in determining chapati quality.

There were some unexpected findings. One was the far higher frequency of the resistant (null) allele of *Tsn1*, which contributes to resistance to nectrotrophic diseases, in Indian compared to Australia cultivars. This will be investigated further by Prof Trethowan in CIM/2005/020. The second was the finding of variation in *Spa-A1* and *Spa-D1* in Indian, but not Australian cultivars. This will be investigated further by Dr Sewa Ram.

Detailed protocols were written by Dr Cane and distributed for many of these genes. As will be discussed later, the Generation Challenge Program has expressed an interest in distributing these protocols more widely than was anticipated in this project.

Ppd-B1 was added in Australia, but as these protocols were obtained in confidence from the John Innes Centre in the UK, no results were shared with our Indian colleagues. These will be distributed if approval is obtained from the John Innes Centre in a reasonable period of time.

4.3 Coordination with CIM/2005/020 'Molecular marker technologies for faster wheat breeding in India'

Activities in this project were closely coordinated with those in CIM/2005/020. Dr Eagles attended the ICIS workshop from 13 to 16 September 2010 in Delhi and Drs Eagles and Cane travelled with Prof Trethowan, Dr Bariana and Dr Brettell in February 2011 and contributed to Dr Brettell's review of CIM/2005/020. During February, Dr Eagles observed the successful use of ICIS in breeding programs in India, in both the GMS (Genealogical Management System) and DMS (Data Management System). This was especially true for the program at Punjab Agricultural University led by Dr Bains.

Prof Trethowan entered the marker results into the ICIS database for subsequent use in both India and Australia.

As relevant pedigrees were identified, these were sent to Prof Trethowan and Mr Manoj Singh for entry into ICIS.

4.4 Presentations

The following presentations were made during this SRA:

Howard Eagles and Karen Cane. Using Diagnostic Markers and Cross Prediction. 15 September 2010, Delhi. Full seminar.

Howard Eagles and Karen Cane. Using Diagnostic Markers and Cross Prediction. SRA: Applying wheat quality markers in India. 6 October 2010, Adelaide. Short presentation. In this presentation plans for the SRA were presented and accomplishments during the first 3 months were described.

Howard Eagles and Karen Cane. Using Diagnostic Markers and Cross Prediction. SRA: Applying wheat quality markers in India. 16 February 2011, Karnal. Short presentation. In this presentation accomplishments of the SRA were described and discussed, especially how the reference set activity could be used in Indian wheat breeding and how Indian

wheat breeders should consider the effects of winter alleles of the *Vrn-A1* gene (Eagles *et al*. 2011) on the performance of their most important cultivars.

Howard Eagles. Association Genetics, ICIS, and Cross Prediction. 30 March 2011, Hyderabad. Full seminar. In this presentation the association genetics approach used in our research was described with emphasis on how ICIS was essential for predictions with minimal bias. The CrossPredictor software developed by the Molecular Plant Breeding CRC was described and the incorporation of similar functionality in future plant breeding software was advocated.

4.5 Important meetings

On 11 September 2010 Dr Cane visited the laboratories of Dr Ratan Tiwari at DWR in Karnal. She concluded that the laboratories in Karnal possessed all the equipment necessary to complete the molecular analysis proposed in this SRA. Advice was offered on the interpretation of molecular marker results already obtained in the laboratory and a short presentation on specific diagnostic marker protocols was given by Dr Cane to Dr Tiwari, research staff and graduate students.

On 15 September 2010 Dr Cane visited the laboratories of Dr Mahendru Singh at IARI; the laboratory capabilities were reviewed and found to be highly satisfactory for the purposes of this project. A short presentation on specific diagnostic marker protocols was presented to Dr Mahendru Singh and her assistant Mr Santosh Singh, who would be responsible for the molecular analysis

On 18 February 2011 discussions were held among Dr Anju Mahendru Singh, Dr G.P. Singh, Dr Karen Cane and Dr Howard Eagles at IARI in Delhi. During these meetings we reached the following important conclusions:

1. The biscuit wheat cultivars had been successfully introduced into India and crosses were being made with elite lines from Dr G.P. Singh's breeding program. There was agreement on the concepts of using diagnostic markers for backcross or topcross enrichment, and Dr Mahendru contributed ideas on potentially superior markers for use in the breeding of biscuit cultivars for India. She is ahead of the Australian collaborators for several aspects of the work. There seemed to be no technical impediment to Drs Mahendru and Singh using advanced molecular breeding techniques to develop biscuit wheat cultivars suitable for northern India, with the question of more precise adaptation to north-western India to be determined as the material is developed and tested.
2. Association genetics, which is the real strength of the long-term research of Drs Eagles and Cane in Australia (for example Eagles et al. 2010), is not a priority for the collaborators at IARI at this time. Therefore, there were no grounds for continuing with a larger project.

4.6 Cross prediction and ICIS

Howard Eagles has long been an advocate of incorporating cross prediction into ICIS, a view that he promoted in Hyderabad on 30 March 2011. On 1 April 2011, in Hyderabad, Dr Graham McLaren of the Generation Challenge Program described plans for an Integrated Breeding Workbench that would include the current data management aspects of ICIS, statistical analyses, and cross prediction. This is a major undertaking with decisions required far beyond the level of this SRA.

4.7 Germplasm of potential value in Australia

While visiting Karnal on 15 September 2011, Drs Eagles and Cane observed breeding activities with the objective of developing cultivars suitable for high pH subsoils. Both India and Australia have soils of this type. We were already aware of the ability of the Indian landrace Kharchia to grow on soils of this type, but during that tour we were told that KRL 19, a derivative of Kharchia, probably contained the same genes as Kharchia for adaptation to these hostile soils. KRL 19 is agronomically superior to Kharchia. KRL 19 has already been introduced into Australia, with seed available from the Australian Winter Cereals Collection. We plan to characterise KRL 19 for known genes and therefore to facilitate its use as a parent in Australian breeding programs.

5 Conclusions and recommendations

5.1 Conclusions

Five elite biscuit cultivars were identified, introduced into India, and crossing to introduce their desirable puroindoline and glutenin genes into adapted Indian breeding lines has commenced. The concept of using diagnostic markers to improve the probability of selecting cultivars with biscuit quality, grain yield and disease resistance required for Indian conditions has been accepted by key Indian wheat breeders. They will probably extend and modify the concepts to suit their own conditions and priorities.

The reference set activity should assist Indian wheat breeders and molecular biologists working with them to increase the range of molecular markers in their breeding programs beyond those already being used in CIM/2005/020.

Part of the reference set activity, particularly protocols for newer markers, has wider application beyond the participants in this SRA.

5.2 Recommendations

Through CIM/2005/020, Indian wheat breeders should be encouraged to extend the use of molecular markers in their wheat breeding programs and to use appropriate markers and protocols from this SRA. The same will occur for Australian wheat breeders through GRDC Project UA00120, that continues to fund the work of Karen Cane and Howard Eagles.

That selected protocols that were largely developed in this SRA, with some overlap with UA00129, be made more widely available through the Generation Challenge Program.

Through the Generation Challenge Program, ACIAR should encourage the development of cross prediction capability in the Integrated Breeding Workbench proposed by Dr Graham McLaren and described in Hyderabad on 1 April 2011.

6 References

6.1 References cited in report

Eagles H.A., Cane K., Kuchel H., Hollamby G.J., Vallance N., Eastwood R.F., Gororo N.N., Martin PJ. 2010. Photoperiod and vernalization gene effects in southern Australian wheat. Crop and Pasture Science 61, 721-730.

Eagles H.A., Cane K.,Trevaskis B. 2011. Veery wheats carry an allele of *Vrn-A1* that has implications for freezing tolerance in winter wheats. Plant Breeding, in press.

6.2 List of publications produced by project

Nil.

7 Appendix

Nil.