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FINAL REPORT 2013

PROJECT CODE : S0610R

PROJECT TITLE
Barley germplasm development phase 2: Evaluation of bulbosum genes and implementation for barley improvement.

PROJECT DURATION

Project Start date	1 July 2010
Project End date	30 June 2013

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PROJECT REPORT

Executive Summary

This project demonstrated that the wild barley species, *Hordeum bulbosum,* possesses new sources of genes that may provide agronomically valuable traits to Australian barley breeding programs. New traits identified include resistance to leaf rust, scald, net form net blotch and tolerance to boron, salt and moisture stress.

Third generation backcross lines of bulbosum leaf rust resistance in a Commander background were provided to the University of Adelaide Barley Breeding Program. Germplasm with potential boron tolerance are also undergoing evaluation by the University of Adelaide Barley Breeding Program.

Germplasm from 8 new bulbosum accessions has been successfully crossed with Commander barley providing a potential source of many new traits for barley breeding.

The challenge remaining to develop this material further is to adopt new genotyping by sequencing technologies to rapidly characterise this material and to allow rapid backcrossing to reduce the quantity of bulbosum DNA in hybrid derived germplasm.

Project Objectives

The project objectives were:

- To test the unique barley genetic resources developed in the SAGIT funded project S0107R for a range of agronomically valuable characteristics, specifically resistance to leaf rust, net form net blotch, scald, crown rot, root lesion nematode, and tolerance to boron, frost, salt and moisture stress.
- To advance the backcross material to a stage which is suitable for crossing into the barley breeding program.

Overall Performance

This project set a very ambitious set of KPIs to maximise the opportunities for identifying useful traits within the barley x bulbosum hybrid germplasm developed at SARDI. Consequently, not all KPIs were met but we now have a very clear idea of the potential value of this germplasm for barley improvement and also have a clear idea of the technologies that will be required to allow these traits to be incorporated into barley varieties.

The project used two streams of barley x bulbosum hybrid germplasm: (1) Advanced backcross germplasm obtained from Dr Richard Pickering (NZ Institute for Crop and Food Research Ltd), and; (2) New hybrid germplasm generated by this and previous projects using Australian barley varieties and new accessions of *Hordeum bulbosum* imported by SARDI.

Leaf rust resistant germplasm identified in the first stream was subjected to 3 recurrent backcrosses to barley variety Commander and given to Drs Jason Eglinton and Stewart Coventry (University of Adelaide Barley Program) for further testing and cultivar development. The second stream of germplasm included new primary hybrids between Commander barley and eight different *H bulbosum* accessions. Useful sources of net form net blotch resistance, scald resistance, moisture stress tolerance, salt tolerance and possibly boron tolerance were identified in this germplasm. Difficulties were encountered in backcrossing the bulbosum primary hybrids into a Commander background because many backcross plant appeared to have either a large amount of bulbosum germplasm or none at all (ie. some backcross progeny resembled barley and did not express the genes of interest from bulbosum). This result was unexpected and without appropriate tools for

rapid genetic analysis, it is very difficult to advance this germplasm quickly for breeding purposes. Consequently the germplasm was not developed to a sufficiently stable state to evaluate its reaction to crown rot, root lesion nematode or frost.

The project was led by Dr Phil Davies and conducted by Dr Parminder Sidhu (SARDI) in collaboration with Drs Jason Eglinton and Stewart Coventry (University of Adelaide), Dr Hugh Wallwork (SARDI) with some input from Prof Mark Tester and Dr Yuri Shavrukov (ACPFG) and Drs Klaus Oldach and Katherine Linsell (SARDI).

Key Performance Indicators (KPI)

KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
1. Meet with Prof Mark Tester and ACPFG scientists to design suitable assay to measure drought response of the bulbosum BC lines. *September 2010*	Yes	
2. Analysis of scald infection using the transgenic *Rhynchosporim* strain 332a expressing the fluorescent GFP protein in collaboration with Dr Klaus Oldach (SARDI). *December 2010*	Yes	
3. Sow single row seed multiplication plots for 5 selected bulbosum BC lines. *May 2011*	Yes	
4. Complete analysis of boron status of selected bulbosum backcross lines in collaboration with Dr Jason Eglinton (University of Adelaide). *September 2011*	Yes	
5. Complete leaf rust, net form net blotch, analysis for selected bulbosum BC lines in collaboration with Dr Hugh Wallwork (SARDI). *May 2011*	Yes	
6. Complete drought response analysis of bulbosum BC lines in collaboration with ACPFG. *April 2012*	Yes	
7. Sow replicated field trials of 5 selected bulbosum BC lines to assess frost and salt tolerance in collaboration with Dr Jason Eglinton. Sow additional single row seed multiplication plots for additional BC lines if necessary. *May 2012*	No	Bulbosum lines have been multiplied in the field by Dr Jason Eglinton's team. However we believe that they are not of sufficient genetic stability to obtain useful data on frost tolerance.
8. Complete crown rot analysis for selected bulbosum BC lines in collaboration with Dr Hugh Wallwork. *December 2012*	No	Due to the large amount of bulbosum germplasm apparent in the hybrid lines it was not possible to do reliable tests for crown rot which requires large numbers of replicates of relatively uniform material.
9. Complete analysis of field data for salt and frost tolerance of BC lines in collaboration with Dr Jason Eglinton. *March 2013*	No	This KPI relied on successful completion of KPI 7 which was not achieved.

10. Complete root lesion nematode analysis for 2 selected BC lines in collaboration with Dr Alan McKay (SARDI). *June 2013*	No	Due to the large amount of bulbosum germplasm apparent in the hybrid lines it was not possible to do reliable tests for root lesion nematode which requires replicates of relatively uniform material.

Technical Information

This project evaluated 17 accessions of *Hordeum bulbosum* for resistance to the diseases leaf rust, net form net blotch and scald and evaluated two advanced backcross lines from Dr Richard Pickering (Crop and Food Research, New Zealand) as candidates for resistance to leaf rust and scald with a view to backcrossing useful genes into an Australian variety. Typical disease resistance reactions are illustrated in Appendix 1, Figs. 1, 2 and 3.

Eight of the 17 bulbosum accessions were successfully crossed to Commander barley to produce fertile F_1 hybrids. Two hybrids plus one of the lines obtained from Dr Richard Pickering were extensively multiplied and evaluated for agronomically useful traits. Additional technical details are summarised in Appendix 1 attached to this report.

The RP76 (Pickering) hybrid lines were advanced to the third backcross generation using leaf rust resistant progeny as parents. High levels of leaf rust resistance were observed among BC_3F_2s. These lines have been given to Dr Jason Eglinton and Dr Stewart Coventry for further evaluation in the University of Adelaide Barley Breeding Program.

The Hb005 hybrid lines were advanced to F_4 and BC_1F_2 and high levels of scald resistance and net form net blotch resistance were observed in later generations. Hb016 hybrid lines were advance to F_3 but the levels of scald resistance and leaf rust resistance diminished with advancing generations. Cytogenetic analysis did not provide sensitive enough data to provide clear understanding about the reasons for these observations.

Experiments were conducted in collaboration with Dr Klaus Oldach and Dr Katherine Linsell (SARDI) to determine whether the scald resistance observed in bulbosum accession Hb005 is that same as that observed in the highly resistant variety Osiris. Hb005, Osiris and hybrid lines were infected with a highly virulent scald isolate, 332a, which had been genetically modified to express the green fluorescence protein to allow microscopic investigation of the infection process. Photographic evidence provided in Appendix 1 (Fig. 4.) could not distinguish between the infection process observed in Osiris and Hb005 so further genetic techniques will be required to determine whether the Hb005 resistance is conferred by a different gene.

It is possible that bulbosum has higher levels of boron tolerance than cultivated barley. Small scale experimentation did not produce conclusive results so F_3 populations of Hb005 x Commander (Appendix 1, Table 6) were provided to the Adelaide University Barley Breeding Program for further glasshouse and field evaluation.

Hydroponic experiments conducted in collaboration with Dr Yuri Shavrukov (ACPFG) using Hb016, Commander and hybrids derived from these parents, indicated that there may be useful sources of salt tolerance in bulbosum. This tolerance appears to be physiological tolerance to sodium accumulation which is different to the sodium exclusion mechanism which exists in the best salt tolerant breeding line, JE001*02D/20 (Appendix 1, Figs. 5, 6, 7 and 8).

Preliminary experiments on water stress tolerance, also conducted in collaboration with Dr Yuri Shavrukov, indicated that bulbosum may have genes which would allow barley to recover better after water stress events. Hb016 bulbosum and Commander x Hb016 germplasm produced higher biomass and better rates of survival after water stress than Commander (Appendix 1, Figs. 9 and 10).

Conclusions Reached &/or Discoveries Made

- *Hordeum bulbosum* is a source of agronomically useful genes for barley breeding.
- Genes of interest include resistance to the diseases scald, leaf rust and net form net blotch and tolerance to the abiotic stresses boron, salt and moisture stress.
- Germplasm from 8 new bulbosum accessions has been successfully crossed with Commander barley providing a potential source of many new traits for barley breeding.
- Third generation backcross lines of bulbosum leaf rust resistance in a Commander background were provided to the University of Adelaide Barley Breeding Program.
- Germplasm with potential boron tolerance are also undergoing evaluation by the University of Adelaide Barley Breeding Program.
- The remaining challenge for developing this material is to adopt new genotyping by sequencing technologies to rapidly characterise this material and to allow rapid backcrossing to reduce the quantity of bulbosum DNA in hybrid derived germplasm.

Intellectual Property

The intellectual property developed in this project is the bulbosum x barley germplasm. It has potential to be used for breeding new barely varieties within the University of Adelaide Barley breeding program.

Application / Communication of Results

Main findings

This project found:

- There are useful sources of disease resistance and abiotic stress tolerance in the wild barley species *Hordeum bulbosum* for barley breeding.
- Genes of interest include resistance to the diseases scald, leaf rust and net form net blotch and tolerance to the abiotic stresses boron, salt and moisture stress.
- It is possible to cross these genes into barley from *Hordeum bulbosum*.
- It is difficult to minimise the quantity of bulbosum DNA in the hybrids rapidly without the adoption of new genotyping by sequencing technology.

Potential industry impact

If these new genes were easily available to barley breeders the impact on the barley industry would be significant. The availability of new resistance sources for scald, leaf rust and net form net blotch would provide greater insurance against these diseases by providing a more diverse array of genetic defences. The availability of boron, salt and moisture stress tolerance would allow more profitable barley production, particularly in more marginal country.

Extension/communication

The target group for this research is barley breeders and researchers and most of the communication has been through meetings with these people and representatives of SAGIT and GRDC. A presentation of this research was given at the 15th Australian Barley Technical Symposium in Adelaide in September 2011.

Suggested path to market

Some germplasm has already been provided to the University of Adelaide Barley Breeding program for development of leaf rust resistant and boron tolerant barley varieties. However the time required for these traits to be released as new varieties is impeded by the unknown quantities of bulbosum DNA which remains in the hybrids and decreases yield potential. Future work is required where genotyping by sequencing technologies are adopted so that the bulbosum DNA can be rapidly decreased.

POSSIBLE FUTURE WORK

Dr Phil Davies and Dr Klaus Oldach are currently drafting a research proposal to develop genotyping by sequencing methods which can be applied to the valuable barley x bulbosum germplasm that has been developed in this project. The new techniques will allow rapid and accurate assessment of bulbosum DNA in hybrid lines to facilitate an effective and timely recurrent backcrossing program.

AUTHORISATION
Name: Dr Kathy Ophel-Keller
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Signature:
Date: 30/1/14

Appendix 1

SAGIT Project S0610R

Barley germplasm development phase 2: Evaluation of bulbosum genes and implementation for barley improvement.

Dr Phil Davies and Dr Parminder Sidhu (SARDI)

Project objectives

The aim of the project was to develop barley germplasm with disease resistance and abiotic stress tolerance genes which are not available using conventional breeding methods and to progress it to a stage which is suitable for crossing into the barley breeding program.

The specific project objectives were:

- To test the unique barley genetic resources developed in the SAGIT funded project S0107R for a range of agronomically valuable characteristics, specifically resistance to leaf rust, net form net blotch, scald, crown rot, root lesion nematode, and tolerance to boron, frost, salt and moisture stress.
- To advance the backcross material to a stage which is suitable for crossing into the barley breeding program.

Summary of results

- This project demonstrated that *Hordeum bulbosum* possesses new sources of genes that may provide agronomically valuable traits to Australian barley breeding programs.
- New traits identified include resistance to leaf rust, scald, net form net blotch and tolerance to boron, salt and moisture stress.
- Difficulties encountered with producing backcross lines from wild bulbosum accessions did not allow tests for crown rot, root lesion nematode and frost tolerance to be conducted.
- Third generation backcross lines of bulbosum leaf rust resistance in a Commander background were provided to the University of Adelaide Barley Breeding Program.
- Germplasm with potential boron tolerance are also undergoing evaluation by the University of Adelaide Barley Breeding Program.
- Germplasm from 8 new bulbosum accessions has been successfully crossed with Commander barley providing a potential source of many new traits for barley breeding.
- The remaining challenge for developing this material is to adopt new genotyping by sequencing technologies to rapidly characterise this material and to allow rapid backcrossing to reduce the quantity of bulbosum DNA in hybrid derived germplasm.

Germplasm and disease evaluation

The project built on the progress of SAGIT project S0107R in which a genotypically diverse collection of 44 *Hordeum bulbosum* lines were sourced from international germplasm banks and advanced backcross lines of barley (Golden Promise) incorporating small segments of the *H bulbosum* genome were provided by Dr Richard Pickering from Crop and Food Research, New Zealand.

In the current project (S0610R), we tested the bulbosum parents for resistance to scald, net form net blotch and leaf rust and the results are summarised in the Table 1. There are very good sources of resistance for all these traits in the bulbosum germplasm.

Table 1 ***Hordeum bulbosum*** **accessions – disease resistance summary.**

Bulbosum Line	Leaf rust	Net form net blotch	Scald (Rs6)	Scald (Rs8)
Hb005	S	R	R	R
Hb007	R	R	R	R
Hb008	R	MR	R	R
HB011	R	R	R	NT
Hb012	R	R	R	NT
Hb013	R	R	R	NT
Hb014	R	MR	R	NT
Hb015	R	MR	R	NT
Hb016	R	MR	R	NT
Hb017	R	R	R	NT
Hb021	R	R	R	NT
Hb022	R	R	R	NT
Hb024	R	MR	R	NT
Hb025	R	R	R	NT
Hb030	R	R	R	NT
Hb033	R	MR	R	NT
Hb034	R	R	R	NT
RP76	R	S	R	NT
RP112	R/MR	MR	MS	NT

R=Resistant; MR=Moderately resistant; MS=Moderately susceptible; S=Susceptible; NT=Not tested.

In project S0107R we generated two primary hybrid crosses with new bulbosum accessions (Hb005 & Hb016) and selected two of the lines from Richard Pickering (RP76 & RP112) for further work. During the course of project S0610R we generated a further 15 primary hybrids between unique bulbosum accessions and barley variety Commander and achieved F_2 seed production in 6 of these hybrids. The full list of barley/bulbosum hybrid lines available is summarised in Table 2.

Table 2. Barley x ***Hordeum bulbosum*** **hybrid germplasm**

	Barley parent	Bulbosum parent	F_1 Hybrids	F_2 seed
1	Commander	Hb005	27	Yes
2	Commander	Hb007	7	Yes
3	Commander	Hb008	10	Yes
4	Commander	Hb013	3	Yes
5	Commander	Hb014	12	Yes
6	Commander	Hb016	3	Yes
7	Commander	Hb024	14	Yes
8	Commander	Hb033	4	Yes
9	Commander	RP76	10	Yes
10	Commander	RP112	20	Yes

Crosses were made to incorporate the new scald, net form net blotch and leaf rust resistance genes into a Commander barley genetic background. Detailed pedigrees are shown in Figs 11, 12 and 13 with a summary of germplasm and disease reactions in Tables 3, 4 and 5 below.

Leaf rust resistant germplasm (10 BC_3F_1 lines) derived from Dr Richard Pickering (Crop and food Research, NZ) and backcrossed into a Commander background were delivered to Drs Stewart Coventry and Jason Eglinton (University of Adelaide Barley program) for further analysis and evaluation for breeding potential. Disease resistant germplasm derived from Hb005 and Hb016 will require further reductions in the quantity of bulbosum genome before they can be utilised by the breeding program.

Table 3. Summary of disease resistance status of Commander x Hb005 germplasm.

Commander x Hb005 Generation	Phenotypes observed		
	Scald (Rs6)	*Net Form Net Blotch*	*Leaf rust*
F_1	R	-	-
F_2	R, MR, MS, S	R, S	-
F_3	R, MR, S *	MR	-
F_4	R, MR, S *	-	-
BC_1F_1	R?, MR, MS, S	MR	-
BC_1F_2	-	MR, S APR: R, S	-

* Also tested with highly virulent scald strain 332a.

Table 4. Summary of disease resistance status of Commander x Hb016 germplasm.

Commander x Hb016 Generation	Phenotypes observed		
	Scald (Rs6)	*Net Form Net Blotch*	*Leaf rust*
F_1	-	-	R
F_2	MR, S	-	MR, S
F_3	MR, MS, S	-	S

Table 5. Summary of disease resistance status of Commander x RP76 germplasm.

Commander x RP76 Generation	Phenotypes observed		
	Scald (Rs6)	*Net Form Net Blotch*	*Leaf rust*
F_1	-	-	-
F_2	R, MS, S	R, S	MR, MS, S
BC_1F_1	-	R, MR, S	-
BC_2F_1	-	R, MR, S	-
BC_3F_1	-	R, MR, MS, S	-

Fig 1. Segregation of bulbosum-derived scald resistance.

Fig 2. Segregation of bulbosum-derived net form net blotch resistance.

Fig 3. Segregation of bulbosum-derived leaf rust resistance.

Analysis of scald infection using the transgenic *Rhynchosporim* strain 332a expressing green fluorescent protein (GFP).

The aim of these experiments, conducted in collaboration with Dr Klaus Oldach and Dr Katherine Linsell (SARDI), was to observe scald infection in barley, *Hordeum bulbosum*, and barley x bulbosum hybrids to determine whether natural resistance observed in the barely variety Osiris had a similar biological mechanism to the bulbosum-derived resistance. A different mechanism of resistance would contribute to a more robust strategy for breeding for scald resistance.

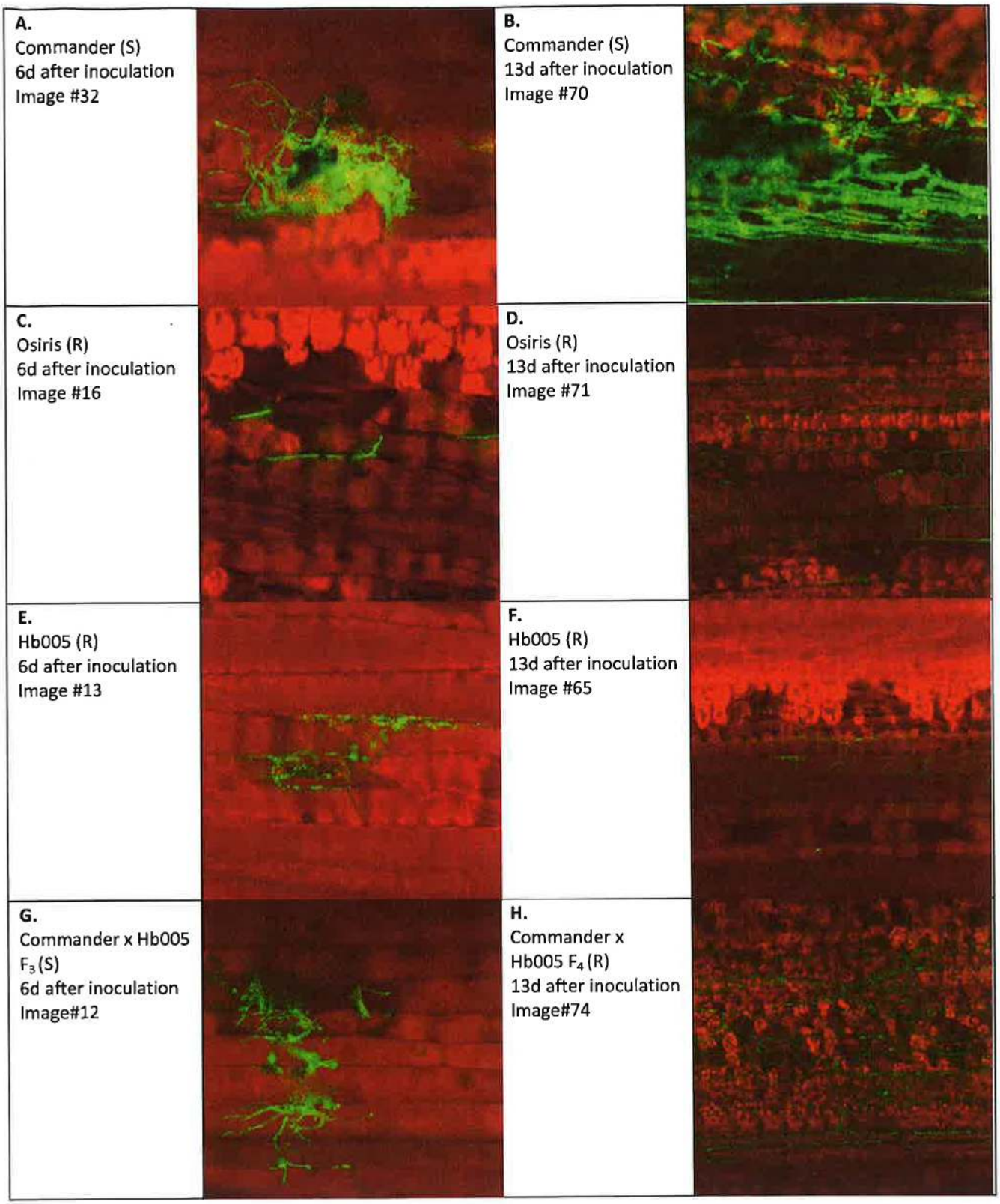
Barley varieties Commander and Osiris, bulbosum accession Hb005 and F_3 and F_4 seed from hybrid lines resistant to scald were planted and screened for scald infection using a highly virulent strain, 332a, which had been engineered to express GFP, allowing it to be easily observed under the microscope. A pictorial summary of the results is presented in the Fig 4 below.

For the susceptible variety Commander, *Rhynchosporim* hyphae were observed to penetrate the cuticle, grow along the junctions between epidermal cells and by 6 days after infection tissue collapse was observed (plates A and B). This was also observed for susceptible segregants from the Commander x Hb005 crosses (plate G).

A similar pattern of hyphal growth was observed for the resistant genotypes Osiris (plates C and D), Hb005 (plates E and F) and resistant segregants from Commander x Hb005 crosses (plate H). This type of analysis did not indicate any morphological differences between the resistance mechanisms operating for Osiris and Hb005. However the genetic and biochemical mechanisms may be different and require additional analysis.

Isolate 332a is an extremely virulent scald isolate with Osiris being one of the few genotypes able to resist infection. It is of great interest that bulbosum line Hb005 carries resistance to this isolate although it is yet to be determined whether this is a different resistance gene(s) to those known in Osiris.

Fig 4. Images of *Rhynchosporium secalis* (Isolate 332a) infecting barley (Commander), *Hordeum bulbosum* (Hb005) and hybrid derived lines 6 and 13 days after inoculation.



Boron tolerance

It is possible that *Hordeum bulbosum* has greater levels of boron tolerance than cultivated barley but preliminary testing of bulbosum germplasm for boron tolerance was inconclusive with laboratory tests. The strategy adopted was to provide populations of hybrid germplasm to the Barley Breeding Program for further field multiplication and testing of populations in the field and glasshouse based 'boron box'.

These experiments are on-going. The populations provided are listed in Table 6:

Table 6. Populations used for boron tolerance evaluation.

Cross	F_2 Plant ID	Population provided	Other traits included
Commander x Hb005	160	F_2	
	552	F_3	NFNB resistance
	553	F_3	NFNB resistance
	595	F_3 and F_4	Scald resistance
	596	F_3 and F_4	Scald resistance
	599	F_3 and F_4	Scald resistance
Commander x Hb005	232	F_2	
Commander x RP76	576	F_3	Leaf rust resistance
	578	F_3	Leaf rust resistance
	580	F_3	Leaf rust resistance
	591	F_3	Leaf rust resistance
	594	F_3	Leaf rust resistance
	622	F_3	Scald resistance
	624	F_3	Scald resistance
	632	F_3	Scald resistance

Salt tolerance

Hydroponics experiments to measure sodium (Na) accumulation in leaves were conducted in collaboration with Dr Yuri Shavrukov (ACPFG). A NaCl concentration of 200mM was used as the salinity stress treatment and compared to a 0mM NaCl control. Single leaves were collected 20 days after applying salt stress and analysed by flame photometry. F_2 and F_3s from a Commander x Hb016 hybrid were compared to barley variety Commander, Hb016 and a doubled haploid line with high salt exclusion capacity, JE001*02D/20.

Fig. 5. Leaf Na and K (mM) following 200mM NaCl treatment

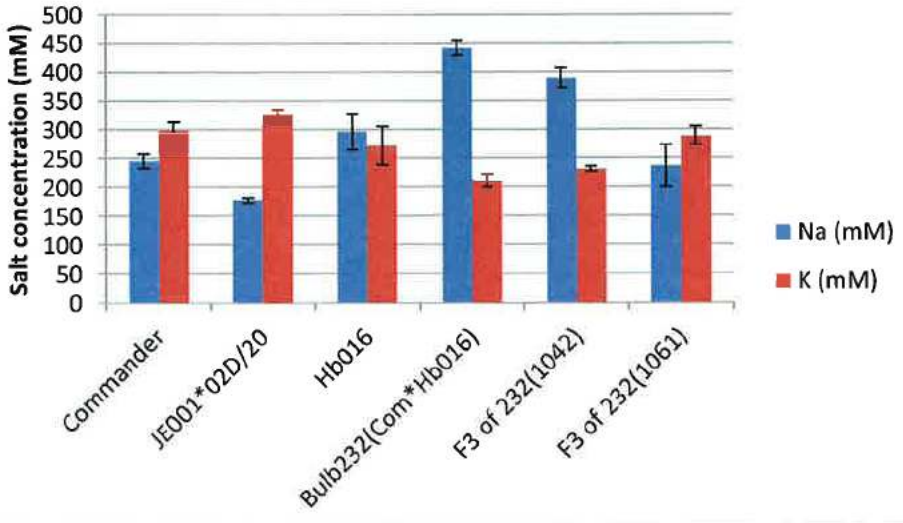


Fig. 5 illustrates the capacity of JE001*02D/20 to exclude sodium from leaf tissue compared to Commander. In contrast, bulbosum line Hb016 and one F_3 population derived from a Hb016 x Commander cross had similar sodium uptake to Commander while the Hb016 x Commander F_2 population and a different F_3 population had increased sodium uptake.

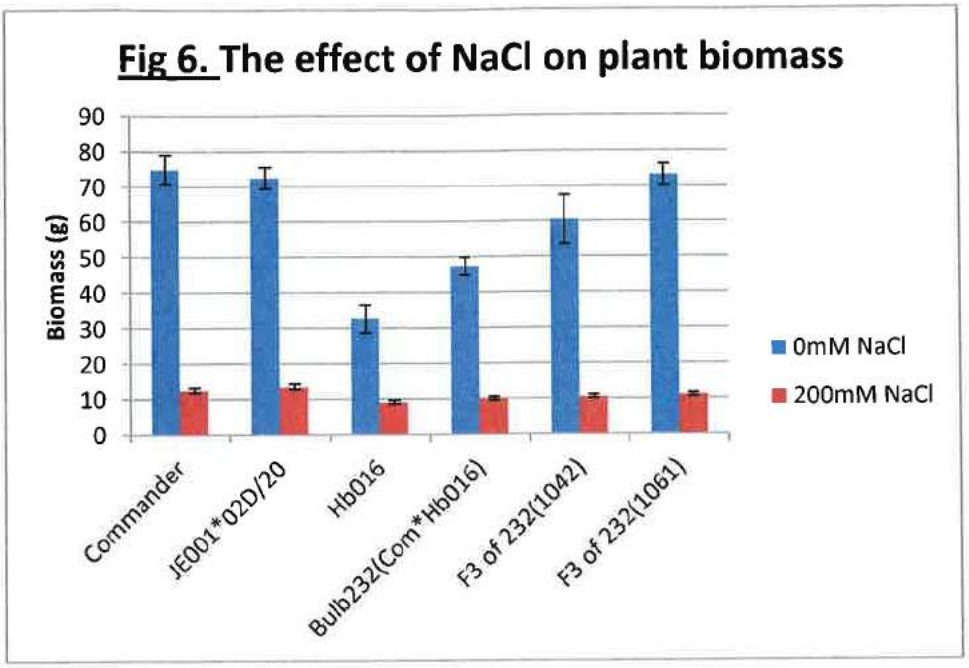


Fig. 6 compares the biomass of the same plants tested in Fig. 5. There is only minimal difference between plant biomass of the various genotypes when treated with 200mM NaCl compared to significant differences without salt stress. Fig.7 shows clearly that bulbosum genotype Hb016 has the greatest relative biomass in under salt stress compared to the unstressed control. This may indicate physiological tolerance of high levels of sodium compared to the exclusion mechanism found in JE001*02D/20.

Further research on the genetics and physiology of this trait may provide alternative sources of salt tolerance for barley breeding.

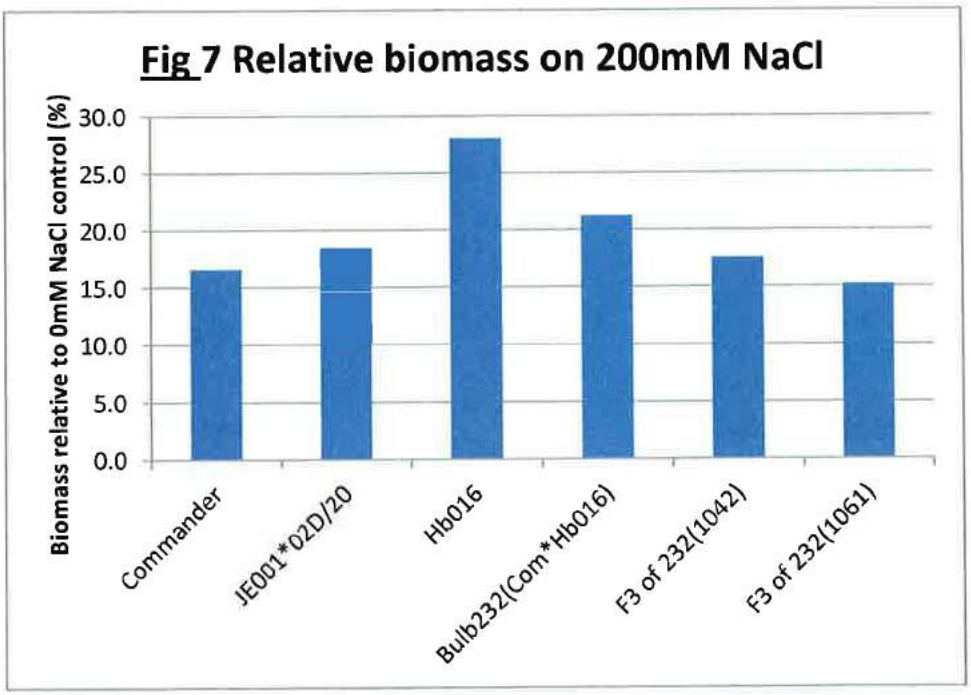
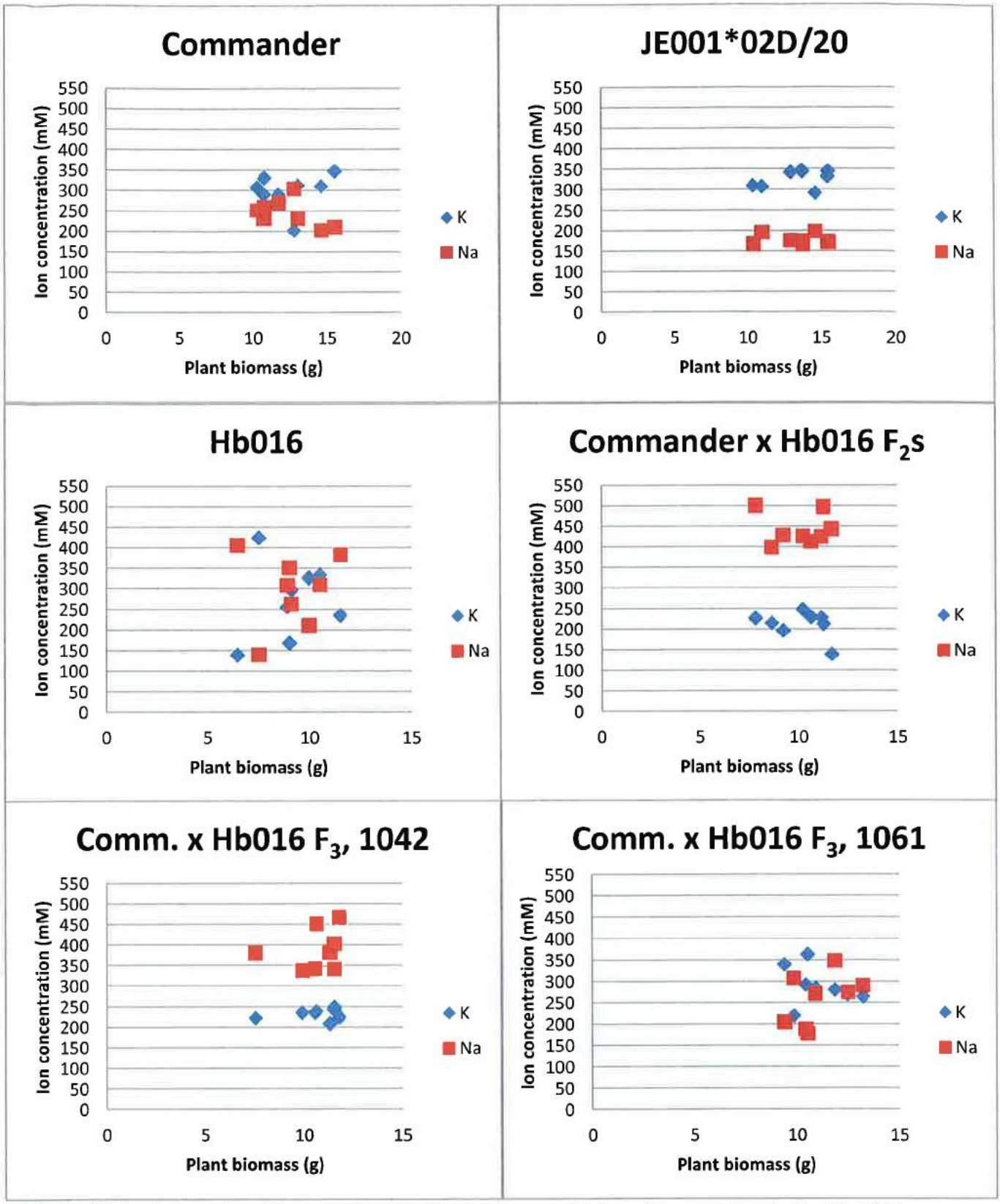


Fig. 8. Individual plant data for experiment summarised in Fig. 5.

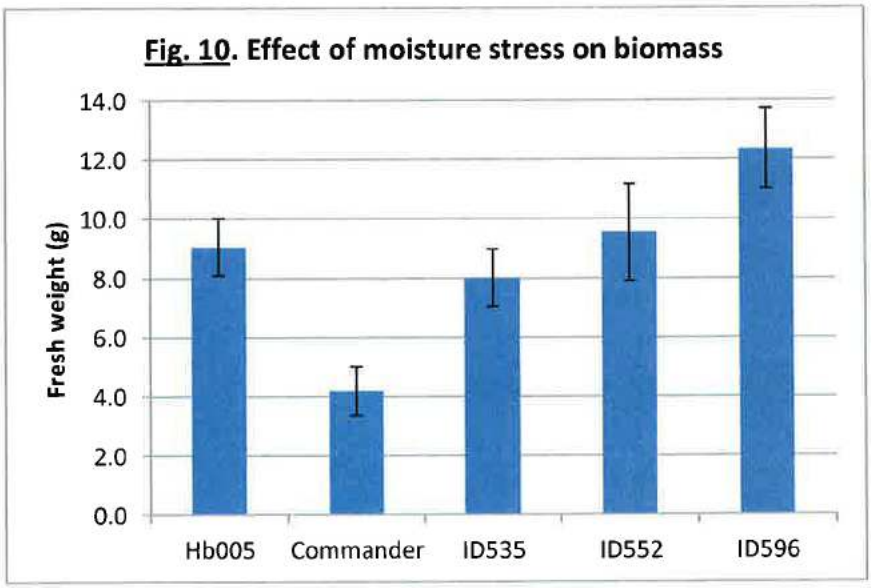


The individual plant data illustrated in Fig. 8 reveal several interesting points. Firstly, Hb016 is genetically variable with some individuals having very high Na and low K content while other individuals are opposite. The Commander x Hb016 F_2s are all derived from F_1 plant ID232 and contain levels of Na between 400 to 500mM with K levels between 200 to 250mM. This is a similar profile to F_3 family 1042. In contrast, F_3 family 1061 which is also derived from F_1 plant ID232 has a similar Na/K profile to Commander. These observations indicate that this is a dominant trait and likely to be a simply inherited. This will be an advantage if it is required for barley breeding.

Water stress tolerance

Preliminary experiments were conducted in collaboration with Dr Yuri Shavrukow (ACPFG) to provide data on water stress response of bulbosum line Hb005, barley variety Commander and progeny of Commander x Hb005 hybrids. The method used compared growth of 5 different genotypes all grown in the same pot under water stress (Fig. 9). Several seeds of each genotype were pre-germinated and seedlings of equal size were planted in each pot. Seedlings were grown with adequate water for 28 days. Water was then withheld for 18 days followed by watering for 11 days and measurement of biomass. The fresh weight of plants grown in 8 replicate pots are summarised in Fig 10.

Fig. 9. Pot assay for water stress tolerance showing plant size after water was withheld.



These preliminary results indicate that Commander produced less biomass following water stress compared with bulbosum line Hb005 and F_3 plants derived from Commander x Hb005 F_2s (ID535, ID552 and ID596). In other experiments where water was withheld for longer than 18 days, Commander plants died but the Hb005 bulbosum lines recovered and continued to grow.

It is clear that bulbosum has a greater capacity to recover from water stress than Commander barley but the genetic and physiological mechanisms of this capacity require further investigation.

Barley x *Hordeum bulbosum* primary cross and selection summary

Fig. 11a. Commander x HbOO5

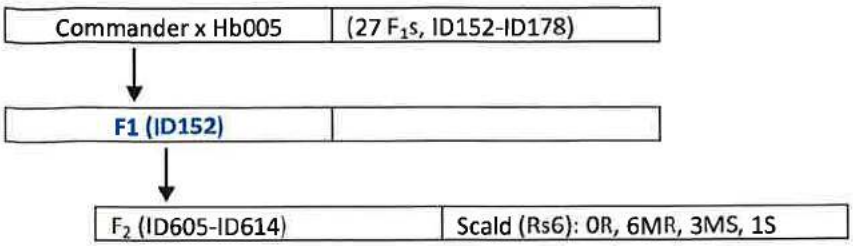


Fig. 11b. Commander x Hb005

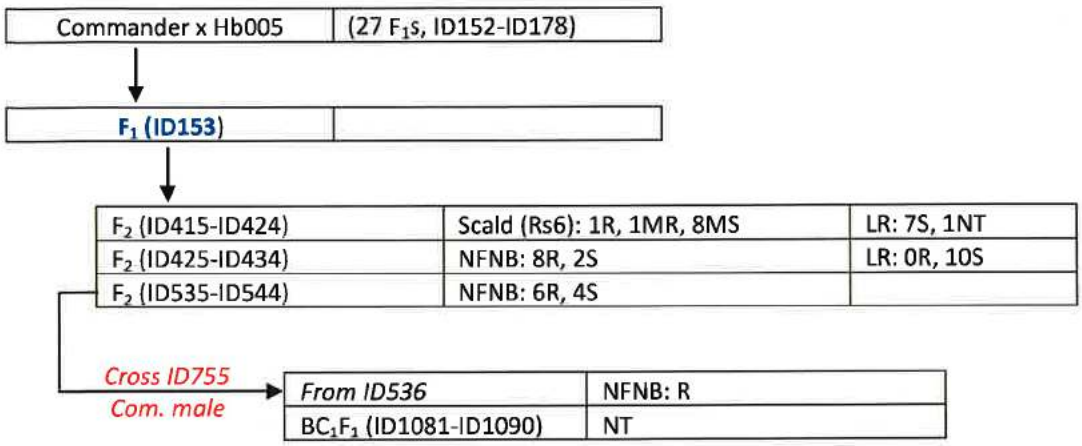


Fig. 11c. Commander x HbOO5

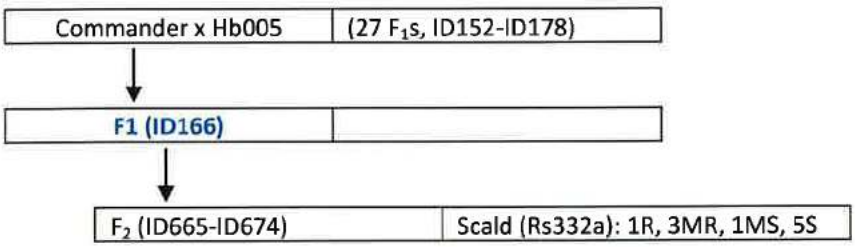


Fig. 11d. Commander x HbOO5

Commander x Hb005	(27 F_1s, ID152-ID178)

↓

F_1 (ID160)	Scald (Rs6): R

↓

F_2 (ID435-ID444)	Scald (Rs6): 5R, 2MR, 2MS	Leaf rust: all S
F_2 (ID445-ID454)	NFNB: 10R, 0S	Leaf rust: all S
F_2 (ID545-554)	NFNB: 6R, 4S	
F_2 (ID595-604)	Scald (Rs6): 3R, 7MR	
F_2 (ID655-674)	Scald (Rs332a): 4R, 4MR, 2S	

→

From ID552	*NFNB: R*
F_3 (ID705-ID714)	NFNB: All MR

	From ID552	*NFNB: R*	
Cross ID756a Com. male →	BC_1F_1 (ID1091)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1092)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1093)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1094)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1095)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 5R, 5S
	BC_1F_1 (ID1096)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1097)	BC_1F_2 NFNB (Seedling): 5MR, 5S	BC_1F_2 NFNB (APR): 1R, 9S
	BC_1F_1 (ID1098)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1099)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1100)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
Cross ID757 Com. female →	BC_1F_1 (ID1111)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1112)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1113)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1114)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1115)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1116)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1117)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1118)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1119)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1120)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S

	From ID553	*NFNB: R*	
Cross ID756b Com. male →	BC_1F_1 (ID1101)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1102)	BC_1F_2 NFNB (Seedling): 10 MR	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1103)	BC_1F_2 NFNB (Seedling): 10 MR	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1104)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1105)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1106)	BC_1F_2 NFNB (Seedling): 10 MR	BC_1F_2 NFNB (APR): 2R, 8S
	BC_1F_1 (ID1107)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 1R, 9S
	BC_1F_1 (ID1108)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1109)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1110)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
Cross ID758 Com. female →	BC_1F_1 (ID1121)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1122)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1123)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1124)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1125)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1126)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1127)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1128)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1129)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S
	BC_1F_1 (ID1130)	BC_1F_2 NFNB (Seedling): 10 S	BC_1F_2 NFNB (APR): 10 S

Fig. 11e. Commander x HbOO5

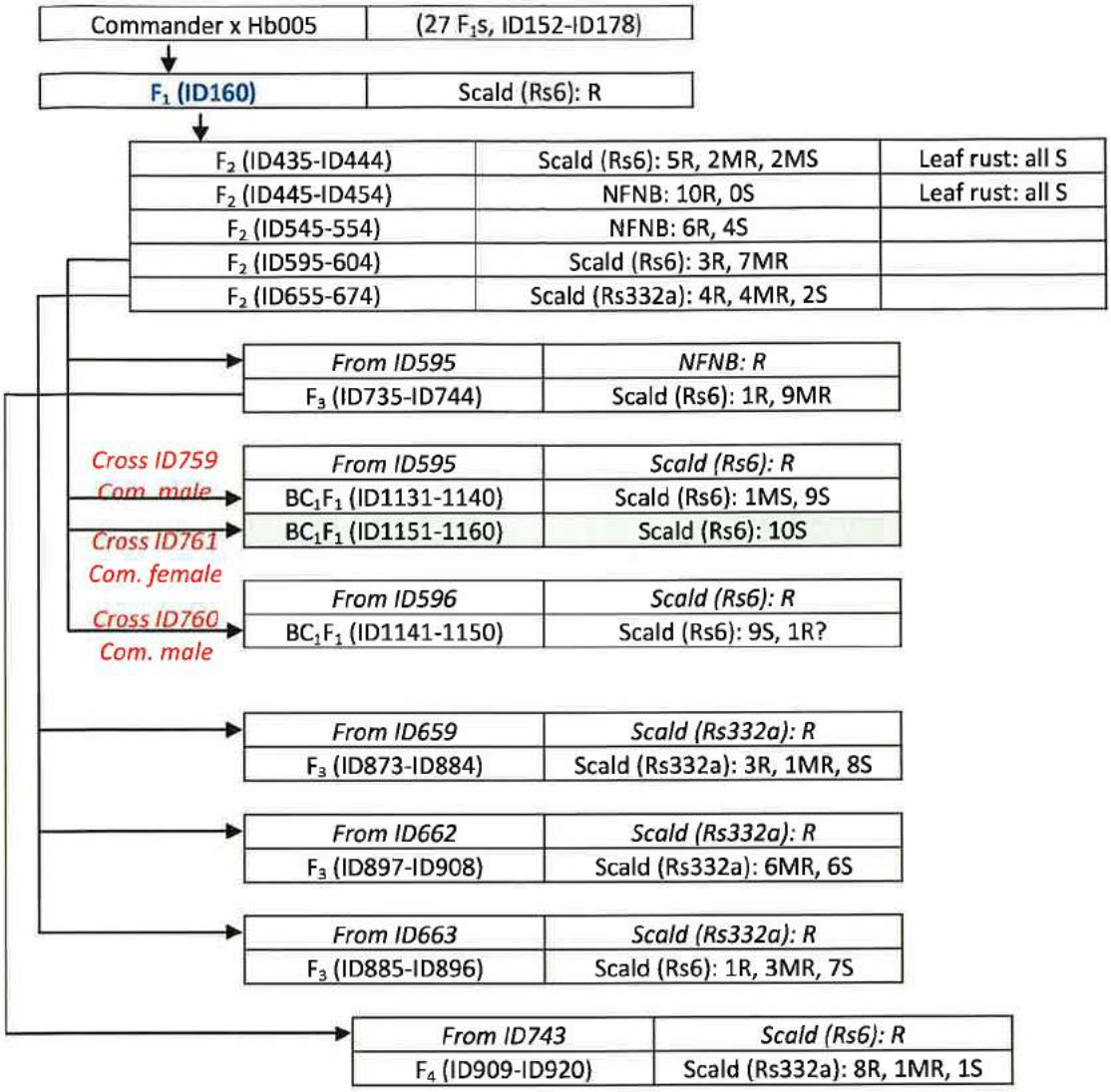


Fig. 12. Commander x HbO16

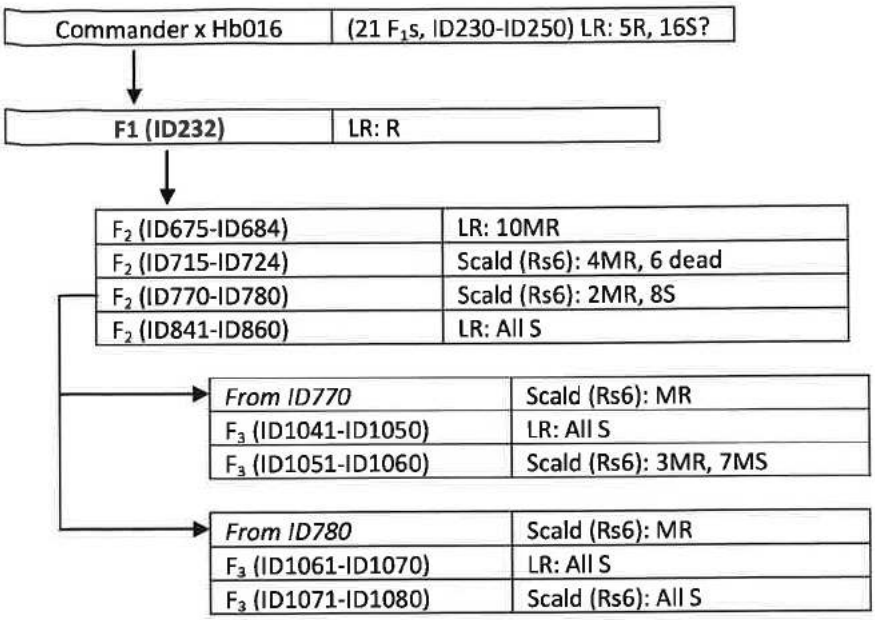
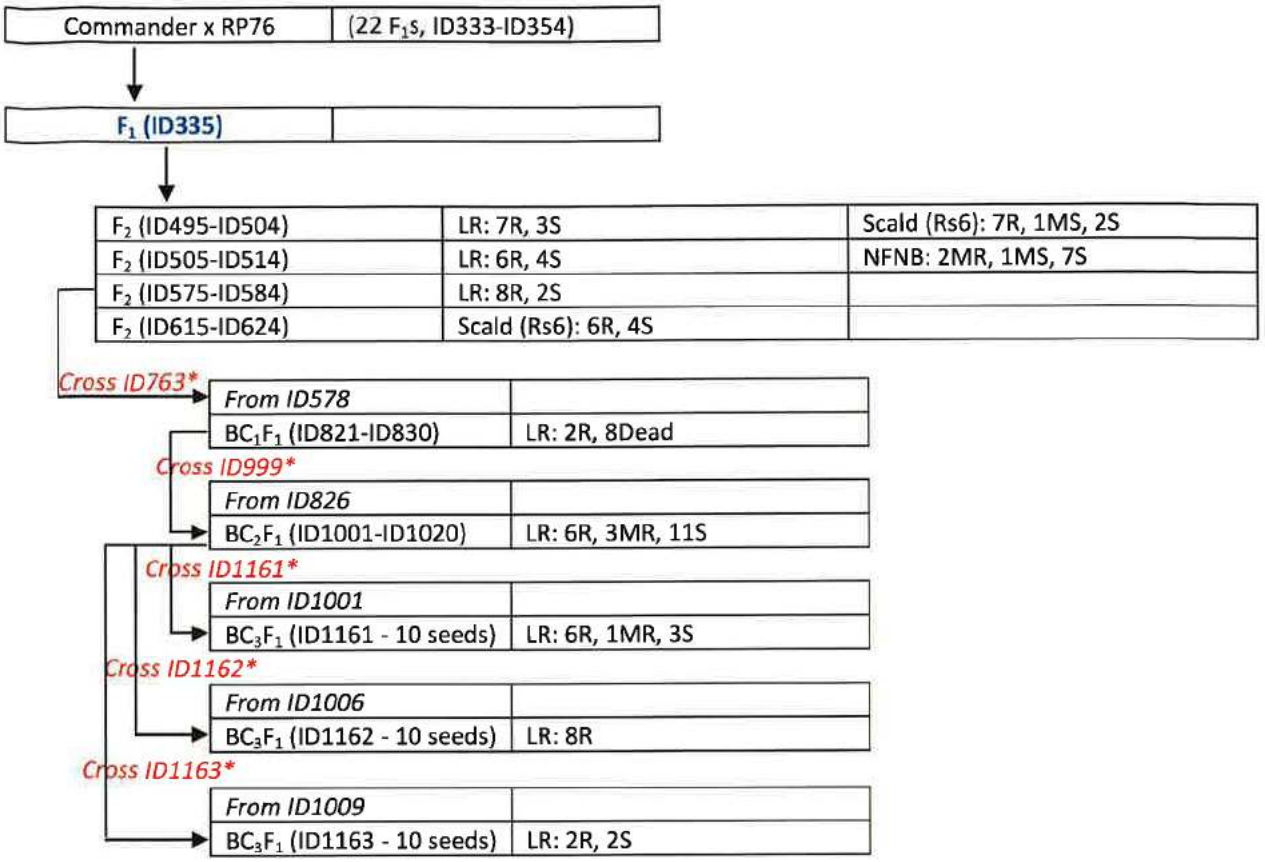
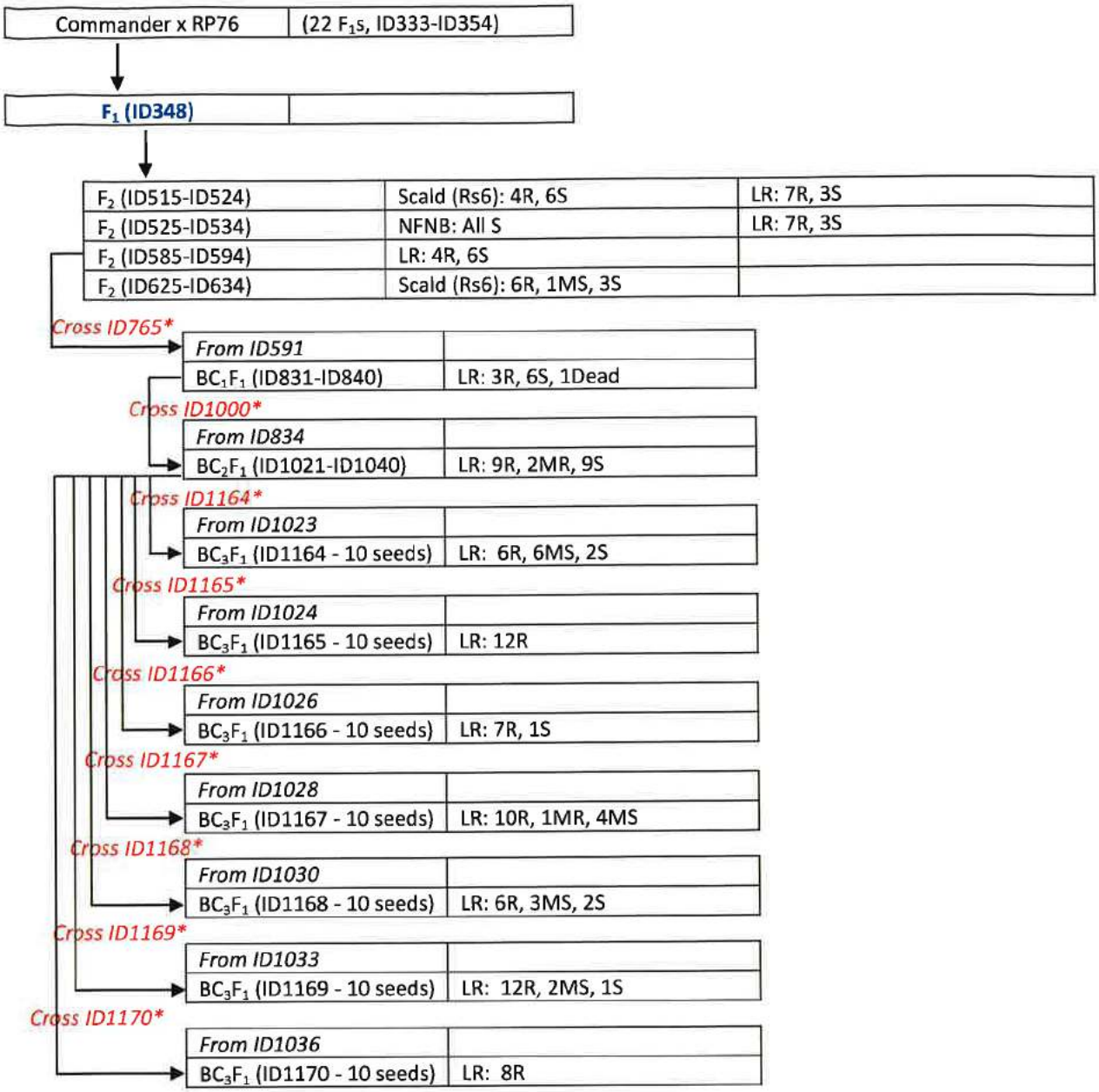


Fig. 13a. Commander x RP76



** Commander as female parent*

Fig. 13b. Commander x RP76



** Commander as female parent*