Seed impurity undermines stripe rust resistance

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Abstract
Sunvale is a popular bread wheat variety throughout central and northern NSW which was commercially released by the University of Sydney in 1995. Significant levels of stripe rust have been reported in commercial Sunvale crops over the past few seasons. However, in National Variety Trials in 2010, a season very conducive to the development of leaf diseases, Sunvale plots had very low levels of stripe rust development consistent with its moderately resistant (MR) rating. This raised the issue as to whether this discrepancy in the commercial versus NVT reaction of Sunvale is related to seed purity, simple variety mix-up or some other factor. Genetic analysis was used to resolve this situation. This analysis compared the DNA molecular marker banding patterns of 20 individual seeds from each of 23 commercial seed lots with the unique pattern only observed in Sunvale. Sixteen of the 23 commercial Sunvale seed lots (70%) contained at least one seed (5%) from a different variety, while seven samples had >10% contamination. The molecular work was complemented by field observations of the various seed lots in a replicated small plot trial at Tamworth in 2011. In 16 of the 20 commercial Sunvale seed lots that had some level of contamination with another variety(s), at least one contaminant was very susceptible to stripe rust. The true Sunvale plants in the plots showed a moderately resistant reaction or better. Results indicate that reported crops of Sunvale with high levels of stripe rust are most likely due to contamination with another more rust susceptible variety.

Key Words
Seed purity, rust resistance, wheat

Introduction
Sunvale is a popular bread wheat variety throughout central and northern NSW which was commercially released by the University of Sydney in 1995. This makes it a 17 year old variety. Although Sunvale relies on the stripe rust resistance gene Yr17, it also has strong Adult Plant Resistance (APR) genes which have allowed it to remain a moderately resistant (MR) variety even to the WA Yr17+ pathotype (134 E16 A+17+), which has virulence for the Yr17 gene (Wellings *et al.* 2012).

Significant levels of stripe rust have been reported in commercial Sunvale crops over the past few seasons. However, in Grains Research and Development Corporation (GRDC) funded National Variety Trials (NVT) in 2010, a season very conducive to the development of leaf diseases, Sunvale plots had very low levels of stripe rust development consistent with its MR rating.

This raised the issue as to whether this discrepancy in the commercial versus NVT reaction of Sunvale is related to seed purity, simple variety mix-up or some other factor. Genetic analysis was used to try and resolve this situation. This analysis compares the DNA molecular marker banding patterns in individual seeds with a unique pattern only observed in Sunvale. The molecular work was complemented by field observations of the various seed lots in a replicated small plot trial at Tamworth in 2011.

Methods
Agronomists were contacted in early 2011 to obtain commercial seed lots of Sunvale from the 2010 harvest. Twenty-three commercial Sunvale seed lots were obtained with 19 coming from northern NSW, 3 from Griffith and 1 from the Wellington region. Seeds from three different Sunvale checks were also included with Sunvale check 1 being the 2011 NVT seed source, while Sunvale checks 2 and 3 were 2006 and 2007 seed sources respectively from Australian Grains Technology (AGT) at Narrabri. Certified seed of three other varieties (LongReach Crusader, Ellison and two different sources of EGA Gregory) were also included to validate the testing.

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In the CSBi laboratories at USQ, DNA was individually extracted from twenty separate seeds from each sample and twelve different molecular markers (on chromosomes 1A, 6A, 1B, 2B, 3B, 4B, 7B x 3 and 2D x 3) were tested across each DNA sample. If the DNA from any single seed did not show the Sunvale banding pattern for one or more of the 12 markers, then it was considered to be another variety. All samples, including the checks, were sent to USQ "blind", identified only by a code.

All thirty seed lots were also sown as small plots (1 x 2 m) at Tamworth in 2011 to obtain detailed observations of head types and stripe rust reaction of individual plants. Four replicate plots of each seed lot were sown in a randomised design with head type and stripe rust reaction recorded for each plot during flowering. Two basic categories of head type were scored being either a 'Sunvale type' which is shorter with a smaller compact head with very little gap between spikelets. This is characteristic of Sunvale and many other of the 'Sun' (bred at Sydney University Narrabri) varieties (e.g. Sunco). The second category was wheat heads that were clearly not characteristic of Sunvale as they were much larger and longer with bigger gaps between spikelets so that the rachis becomes quite visible between spikelets. Other notes on crop height and presence of awns were also taken. Stripe rust was scored for each plant type in each plot on a standard 1-9 visual scale developed by the National Rust Laboratory at Cobbitty (Wellings and Bariana 2004).

Results

The seed samples from two of the three Sunvale checks contained only Sunvale seed (Figure 1). These two Sunvale checks were kindly supplied by Dr Mequin Lui from AGT at Narrabri. The Sunvale check 1 was seed used in the 2011 NVT trial network and appeared to contain a low level of contamination with one other variety. Because only twenty seeds were tested from each sample (due to cost issues) there is still the possibility that a low level of contamination, generally <5%, is present in any of the samples (commercial or checks).

Purity of commercial Sunvale seed lots based on marker analysis varied dramatically with only 7 of the 23 (30% of samples) appearing to be >95% Sunvale (Figure 1). The remaining 16 seed lots were contaminated with at least one other variety. Impurity levels were very high in some commercial seed lots with Sunvale 17 only having 13 of the 20 seeds (65%) identified as true Sunvale while the remaining seeds appear to consist of four different varieties. In the sample Sunvale 6 only 2 of the 20 seeds tested (10%) were Sunvale based on genetic testing. Sunvale 6 seeds appeared to consist of 14 different varieties based on marker analysis. The Sunvale 15 sample contained no Sunvale seed with the molecular marker banding patterns indicating that the seed lot was composed of only one different variety with a pattern consistent with EGA Gregory. This result is probably due to a silo mix-up either at seed preparation for sowing or at harvest.

The field evaluations of head type and rust reaction were very consistent with the marker analysis. Field observations allowed detection of impurity levels <5% in commercial seed lots 5, 13, 14 and 16. Only seed lots Sunvale 4, Sunvale 7 and Sunvale 12 (9% of seed lots) appeared to be pure Sunvale based on molecular and field evaluations. Within 16 of the 20 commercial Sunvale seed lots that had some level of contamination with another variety(s), the contaminant was very susceptible to stripe rust (MS to VS) while the true Sunvale plants in the plots had an MR or better reaction. Of the remaining four seed lots, the contaminant varieties in sample 9 both had Sunvale like heads with possibly a slightly more susceptible MR-MS reaction to stripe rust. Thus the contamination would not be obvious in the paddock due to the similarity in head appearance and rust reaction and only became evident upon molecular analysis. In fact, the contaminants in sample 9 only varied from Sunvale for markers on chromosome 2D, indicating that the contaminants are closely related to Sunvale, possibly a sister line.

Sample 10 appeared to have two contaminant varieties with the main one having an R-MR reaction to stripe rust while the lower frequency contaminant showed an MS-S reaction. Sample 15 had a consistent 'non-Sunvale' head type and an R reaction to stripe rust. The head type was similar to the two EGA Gregory check samples, supporting the marker analysis that this was a straight varietal mix-up. The dominant contaminant in sample 17 based on head type appears to be a durum wheat variety with an MR reaction to stripe rust.

Marker analysis indicated that the NVT Sunvale seed source (Sunvale check 1) contained a 5% impurity. This was also supported by the field observations with an average of 5% of scattered plants having a larger 'non-Sunvale' head type with an MS-S reaction to stripe rust. The field observations also supported the

marker analysis with the Sunvale 6 seed lot. The exception being that the markers indicated that there are 14 different contaminant varieties but only five could be easily distinguished with field observations, including one awnless variety.

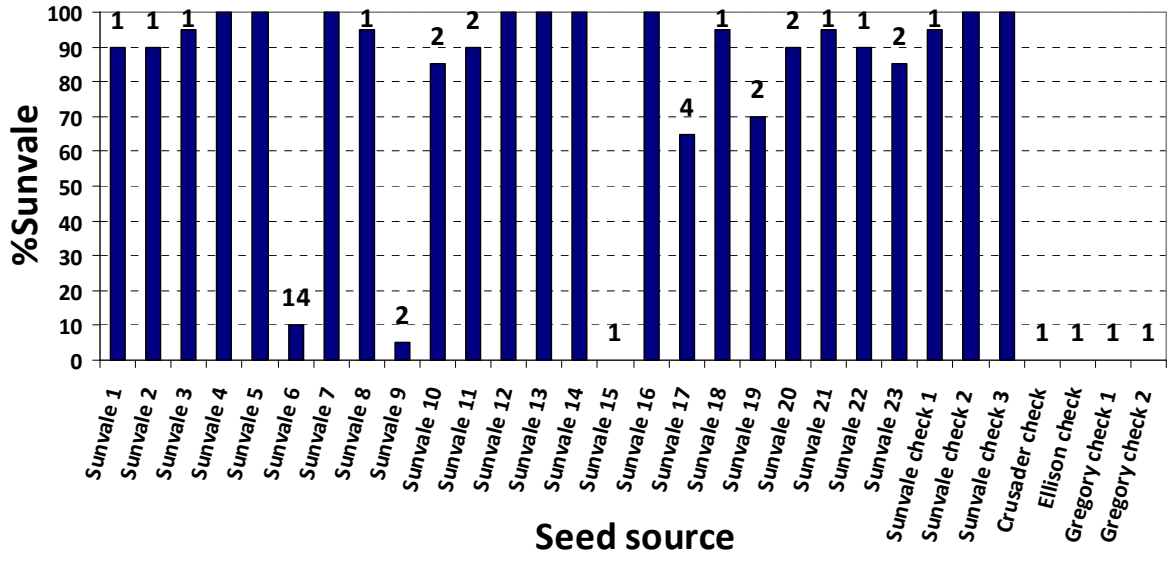

Figure 1: Purity of commercial cv. Sunvale sources in 2011
(Value above bars indicates the number of different contaminant varieties identified by marker analysis)

Conclusion

Impurity (variety contamination) appears quite common in commercial Sunvale crops. If the Sunvale seed happens to have been contaminated with a stripe rust susceptible variety, as evident in 16 of the commercial seed lots, then this would explain the perceived increased stripe rust susceptibility in commercial crops. That is, they are not pure Sunvale, but have been contaminated with another more rust susceptible variety. Straight variety mix-ups (e.g. Sunvale 15) also appear to be an issue.

It is not surprising that high levels of impurity were observed in commercial Sunvale lots given that this is a 17 year old variety. Growers need to take more care in ensuring variety purity and correct identification of seed lots for planting. This study also emphasises the value of growers conducting careful observation of head type and the pattern of disease distribution in crops which are showing unexpected disease reactions. Pure Sunvale remains MR to stripe rust and does not require in-crop fungicide management. However, contamination of commercial Sunvale seed lots has introduced more stripe rust susceptible varieties into these paddocks in most situations which is unfairly tarnishing the resistance rating of this variety and jeopardising industry confidence in stripe rust resistance breeding as a whole. This is the first report of seed impurity being determined as the cause of unexpected stripe rust responses within a wheat variety. This situation is unlikely to be unique to Sunvale and may also explain mixed reports of stripe rust levels commercially in more recently released MR varieties such as EGA Gregory.

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