







REGISTRATION

Germplasm

Registration of the “Woodies” multi-rust-resistant barley germplasm

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Abstract

Selection for resistance to plant diseases is a continuous effort on the part of plant breeders. Sources of genetic resistance are often limited, despite considerable discovery efforts. Stem rust and stripe rust are two diseases of particular importance in barley (*Hordeum vulgare* L.) production. The present work aims to develop and deploy genotypes with resistance to these diseases that can be used in future breeding efforts. The Woodies, Woody-1 (DH160733; Reg. no. GP-218, PI 704479) and Woody-2 (DH160754; Reg. no. GP-219, PI 704480), are two doubled-haploid genotypes produced via F₁ anther culture named in honor of the late Lynn “Woody” Gallagher. These two-row spring habit barley germplasm accessions were released by the Oregon Agricultural Experiment Station in 2023. These genotypes have demonstrated resistance to both stem and stripe rust at the seedling and adult plant stage in trials conducted between 2018 and 2023. The genetic basis of this resistance appears to be a novel quantitative trait locus conferring resistance to both diseases on chromosome 5H that is different from the known *rpg4/Rpg5* complex for stem rust resistance found on the same chromosome. Seed can be requested from the Oregon State University Barley Breeding Program or from the NLGRP Germplasm repository.

1 | INTRODUCTION

Stem rust (caused by *Puccinia graminis* f. sp. *tritici*) and stripe rust (caused by *Puccinia striiformis* f. sp. *hordei*) are two of the most important diseases affecting barley (*Hordeum vul-*

gare L.) production worldwide (Dean et al., 2012). These diseases can have a significant negative impact on barley yield and malting quality through reductions in kernel components such as plumpness, weight, and germination (Chen & Kang, 2017; Roelfs & Bushnell, 1985; Steffenson, 1992). The stem rust race TTKSK (isolate Ug99) and its variants are a serious threat to wheat (*Triticum aestivum* L.) and barley production worldwide (Singh et al., 2015; Steffenson et al., 2017). The

Abbreviations: AMBA, American Malting Barley Association; QTL, quantitative trait loci.

rpg4/Rpg5 complex confers resistance to TTKSK in barley (Steffenson et al., 2009; Zhou et al., 2014). This complex contains three tightly linked genes, *Rpg5*, *HvRga1*, and *HvAdf3*, that are inherited as a single unit. Markers targeting polymorphisms in *Rpg5* have been used to introgress this complex into different elite barley genotypes (Derevnina et al., 2014; Sharma Poudel et al., 2018). However, this complex appears to interact with other genes when it is introgressed into different genetic backgrounds (Hernandez et al., 2019).

Barley stripe rust is most problematic in production areas with cool wet weather, where it can cause yield losses of up to 70% (Chen & Kang, 2017; Chen et al., 1995; Line, 2002). After its initial detection in Colombia in 1975 (Dubin & Stubbs, 1986), the disease spread throughout the Americas, reaching the United States in 1991. Since then, the disease has become a constant threat to barley production (Chen et al., 1995; Marshall & Sutton, 1995; Roelfs et al., 1992). Breeders can deploy genetic resistance using major effect resistance genes, of which at least 26 different ones are known (Chen & Line, 2003; Line & Chen, 1999). Additionally, numerous other studies have identified and mapped quantitative trait loci (QTL) for resistance at the seedling and adult plant stages (Belcher et al., 2018; Castro et al., 2003; Esvelt Klos et al., 2016; Gutiérrez et al., 2015). The most frequently reported QTL are on chromosomes 1H, 4H, and 5H. Recent work has reported adult plant resistance to both stem and stripe rust, resulting from a QTL on chromosome 5H in a mapping population, that is not due to *Rpg5* (Hernandez et al., 2020a).

The Woodies, named in honor of the late Dr. Lynn “Woody” Gallagher, a pioneering developer of disease-resistant barley germplasm at the University of California (Davis), are two doubled haploids (DH160733 and DH160754) known as Woody-1 (Reg. no. GP-218, PI 704479) and Woody-2 (Reg. no. GP-219, PI 7024480). Both genotypes are rough-awned, hulled, two-row, and spring-habit barleys with long rachilla hairs. Both accessions were released by the Oregon Agricultural Experiment Station in 2023. The Woodies were derived via anther culture from the F₁ of the cross DH140512/UC1322. DH140512 is a two-row experimental selection from the Oregon State University barley program with the pedigree SH98076/‘Full Pint’ (Verhoeven et al., 2011). UC1322 is a two-row experimental selection from the University of California (Davis) barley program with the pedigree Z05500120/CIMMYT7862. From 2018 to 2023, the Woodies were tested in various stem rust and stripe rust resistance screening trials. These two sister genotypes were released to the international barley research community as a resource for the introgression of resistance to barley stem rust and barley stripe rust.

Core Ideas

- The Woody-1 and Woody-2 germplasms are novel barley genotypes released by Oregon State University.
- The Woodies are doubled haploid genotypes developed via F₁ anther culture.
- The Woodies are a source of both stem and stripe rust resistance.

2 | METHODS

2.1 | Doubled haploid production and genotype selection

The cross between DH140512 and UC1322 was made in 2015 at Oregon State University, and in 2016 the doubled haploids were produced via F₁ anther culture following the protocol developed by Cistué et al. (2003). The Woodies were part of a set of 384 doubled haploids developed as part of the Cycle II population (<https://barleyworld.org/ug99>). As described in detail in Hernandez et al. (2020a, 2020b), the Cycle II population was developed from crosses between sources of resistance to stem rust race TTKSK identified in Cycle I (Hernandez et al., 2019). DH140512 has the resistant allele at *Rpg5* and resistance alleles at QTL on chromosomes 5H and 7H, whereas UC1322 does not amplify for either the resistance or the susceptibility allele at this locus; it was chosen as a parent due to reported resistance to stem rust in multiple field trials in Africa (L. Gallagher, personal communication, January 18, 2020). As reported by Hernandez et al. (2020a), resistance of both parents at the seedling stage was confirmed and validated at the adult plant stage in a field trial in St. Paul, Minnesota during the 2018 season using race QCCJB as a surrogate for race TTKSK.

2.2 | Phenotyping

Stem and stripe rust data on the Woodies was collected in 19 total environments between 2018 and 2023 (Table 1). This includes locations in Kenya, Ethiopia, Minnesota, Washington, Oregon, and California. It also includes data on adult plant and seedling resistance to stem rust in addition to adult plant resistance to stripe rust; however, each phenotype was not assessed in each environment. Table 1 shows a summary of what phenotypes were assessed in which environments.

TABLE 1 The 19 environments considered in this study and the phenotypic information collected in each environment.

Environment	Code	Stem rust seedling	Stem rust adult	Stripe rust adult
Kenya 21	KNY21		X	
Kenya 22	KNY22		X	
Kenya 23	KNY23		X	
Ethiopia 21	ETH21		X	
Ethiopia 22	ETH22		X	
Ethiopia 23	ETH23		X	
Minnesota 18	MN18	X	X	
Minnesota 19	MN19		X	
Minnesota 21	MN21	X	X	
Minnesota 22	MN22	X	X	
Minnesota 23	MN23	X		
Washington 21	WA21		X	
Washington 22	WA22		X	
Washington 23	WA23		X	
Oregon 19	OR19			X
Oregon 21	OR21			X
California 18	CA18			X
California 19	CA19			X
California 21	CA21			X

Seedling stem rust resistance scores were taken in St. Paul, MN, in 2018, 2021, 2022, and 2023. The Stakman qualitative infection scale was used to describe infection types (Stakman et al., 1962), and the modified Cobb scale was used for disease severity on a 0%–100% basis (Peterson et al., 1948). In 2018, seedlings were tested against race QCCJB as a surrogate for Ug99. In 2021–2023, race TTKSK was used. The 2018 experiment was replicated a single time, whereas the 2021–2023 experiments were each done in two replicates.

Adult plant resistance to stem rust was assessed in a total of 13 environments. These include Njoro, Kenya, between 2021 and 2023; Debre Zeit, Ethiopia, between 2021 and 2023; St. Paul, MN, in 2018, 2019, 2021, and 2022; and Pullman, WA, between 2021 and 2023. Percent severity of stem rust was collected in all locations using a modified Cobb scale (Peterson et al., 1948). Infection response was also recorded in all environments with the exception of Minnesota 2018 and 2019. Infection types included resistant, moderately resistant, moderately resistant to moderately susceptible, moderately susceptible, and susceptible. The Kenya and Ethiopia trials were conducted with replicated check genotypes, the Minnesota trials were replicated twice, and the Washington trials were done in three replicates. More information on the Africa stem rust field trials can be found in Kosgey et al. (2021).

Adult plant resistance to stripe rust was assessed in a total of five environments. These include Corvallis, OR, in 2019 and 2021 and Davis, CA, in 2018, 2019, and 2021. Percent severity was again recorded based on the modified Cobb scale

(Peterson et al., 1948). The Oregon and California trials were replicated twice in 2018 and 2019; the 2021 trials in these locations used only replicated checks. More information on the field trials in Africa where stripe rust data were collected can be found in Kosgey et al. (2021).

Agronomic characteristics of the Woodies were assessed in the 2023 Oregon State Extension Spring Barley Variety Trials. In these trials, grain yield, plant height, test weight, and grain protein as a percentage were recorded in five locations in the state of Oregon during the 2023 season. Testing locations included Athena, Ione, La Grande, Pendleton, and Klamath Falls. There were 18 advanced barley cultivars assessed at each of the experimental locations, with the exception of Klamath Falls, where only 13 could be assessed due to severe bird damage. Additional information on these trials can be found at <https://cropandsoil.oregonstate.edu/wheat/variety-trials/2023-oregon-wheat-and-barley-yield-trial-data>. Additionally, seed increases were grown in Corvallis, OR, during the 2022 season, where heading date was observed compared with commercially available lines and recorded as days since January 1.

Malt quality characteristics were assessed at the Hartwick College Center for Craft Food and Beverage in Oneonta, NY, in January 2023 using grain harvested in the 2022 season Corvallis, OR, seed increases. The American Society of Brewing Chemists Barley methods were used to collect malt quality phenotypes, including fine extract, color, β -glucan content, protein, soluble/total protein, free amino nitrogen, diastatic

power, α -amylase content, plump grains, and thin grains (ASBC, 2023). The measurements were taken using a single replicate. These values were compared with the American Malting Barley Association (AMBA) malting barley breeding guidelines to assess the usability of these genotypes for malting and brewing (AMBA, 2021).

2.3 | Statistical analyses

All statistical analyses were conducted using R version 4.1.3 (R Core Team, 2023). For fully replicated trials, the genotypic means were calculated using a mixed model fit in the lme4 R package (Bates et al., 2015). The model accounted for genotypic and blocking effects following Equation (1):

$$y_{ij} = \mu + G_i + \beta_j + \varepsilon_{ij} \quad (1)$$

where y_{ij} is the phenotypic observation of the i th genotype in the j th environment; μ is the overall mean; G_i is the effect of the i th genotype; β_j is the random effect of the j th replication, with $\beta_j \sim N(0, \sigma_{\beta}^2)$; and ε_{ij} is the error term, with $\varepsilon_{ij} \sim N(0, \sigma_{\varepsilon}^2)$. The terms σ_{β}^2 and σ_{ε}^2 represent the replication and error variance, respectively. In the case of trials with only repeated checks, the replication effect was removed from Equation (1). An ANOVA was conducted using the lmerTest R package (Kuznetsova et al., 2017) to determine the significance of the genotypic main effect. A post hoc analysis was conducted using lmerTest to test the pairwise comparisons of genotypes using Tukey-adjusted p values. This allowed for the construction of compact letter displays and for general assessments to be made about the significance of differences between genotypes.

Broad-sense heritability was also estimated following the methodology proposed by Cullis et al. (2006). This better accounts for missing data and partial replication following Equation (2):

$$H^2 = 1 - \frac{\bar{v}_{\Delta}^{\text{BLUP}}}{2\sigma_g^2} \quad (2)$$

where H^2 is the estimated broad-sense heritability, $\bar{v}_{\Delta}^{\text{BLUP}}$ is the average standard error of genotypic BLUPS calculated in a random-effects model following the same formulation as Equation (1), and σ_g^2 is the genotypic variance.

3 | CHARACTERISTICS

3.1 | Seedling stem rust resistance

Results from the seedling stem rust resistance trials are provided in Table 2. The Woodies and their parents primarily

TABLE 2 Seedling resistance to stem rust following the Stakman qualitative scale.

Genotype	2018	2021 ^a	2022 ^a	2023 ^a
DH140512	0;	13–/11+;	11+;/1+2	11+;/1
UC1322	0;	11+;/11+	1+2;/11+;	1;/1;
Hiproly	3	3–/1/23–	2+;/32+	13–/13–
Full Pint	–	11+;/1+2–	11+2–/3–1+	11+;/11+
Q21861	00;	;1/1;	;1/1;	1;/;
Woody-1	0;1–	11+3–/12	22+;/3–1+	1/1;
Woody-2	0;	11+3–/11+	2;/1+	11+;/1;

Note: Race QCCJB was used in 2018. Race TTKSK was used in 2021–2023. Infection types are listed in their order of prevalence on the leaves.

^aReplications separated by “/”.

displayed a nearly immune infection type (0; to 0;1–) in the 2018 trials, with Woody-1 showing a minority of leaves with small sporulating pustules. Susceptible check ‘Hiproly’ (Helm et al., 1974) displayed a high infection type of 3. In additional experiments with race TTKSK from 2021 to 2023, the Woodies exhibited predominantly low infection types (mostly types 1 and 2), with some higher types (i.e., 3–) observed in low frequency. In one case, a replicate of Woody-1 in 2022 showed a high infection type of 3– as the most common, but type 1+ was also present.

3.2 | Adult stem rust resistance

Results from the adult plant stem rust resistance trials are provided in Table 3. Severity values for Woody-1 ranged from 0% to 37% and were at or below the trial median severity in all environments but WA23. Severity values for Woody-2 ranged from 0% to 27% and were at or below the trial median severity in all environments but KNY23. The susceptible check Hiproly exhibited a severity ranging from 5% to 50% in the 10 environments it was assessed, being at or over the median severity score in all 10 environments. A full table of stem rust severity values for each genotype tested in each trial can be found in Supplemental Table S1, which also includes the standard error and within-environment grouping.

Despite resistance previously displayed in the Woodies under both field and controlled greenhouse conditions, adult stem rust resistance was not apparent during the Washington 2022 field trial. The 2022 field season coincided with a record high heat dome across the Pacific Northwest. Many stem rust resistance genes in wheat are known to be temperature sensitive, such as *Sr6* (Leonard & Szabo, 2005), *Sr15* (Gao et al., 2019), *Sr43* (Yu et al., 2023). Lines carrying these genes usually exhibit higher infection types at elevated temperatures. In barley, *rpg4/Rpg5* is known to be temperature sensitive (Jin et al., 1994). Despite the presence of additional

TABLE 3 Adult plant resistance to stem rust assessed in 14 field environments, including five locations and 6 years. Data include the presence or absence of the *Rpg5* resistance allele, a percent severity score based on the modified Cobb scale, and an indicator of infection type. Trial median severity and broad-sense heritability are also provided.

Genotype	<i>Rpg5</i>	KNY21	KNY22	KNY23	ETH21	ETH22	ETH23	MN18 ^a	MN19 ^a	MN21	MN22	WA21	WA22	WA23
Woody-1	0	1MS	5RMR	0	1MS	10MR	4	6	10MS	3MR	5MR	27SMS	37MS	
Woody-2	0	0	20MRMS	1MS	1MS	15MR	0	7	10MS	0	7MR	27SMS	12R	
DH140512	X	0	1MR	–	5MR	5MR	20MSMR	9	45	23S	–	18S	–	–
UC1322		10MS	1MS	15MRMS	1MS	10MS	20MRMS	5	28	33S	–	30S	–	–
Q21861	X	0	1	–	2MS	13MSMR	6MR	–	–	35S	5MR	43MS	37S	47MS
Full Pint	X	0	0	15MSMR	20MSS	35MSS	50MS	–	–	45S	15MS	46S	47S	50S
Hiproly		10MS	13MS	28MS	20MSS	43S	50SMS	–	–	45S	18MS	83S	43S	–
Median		1	5	15	10	20	30	15	38	37	18	47	43	33
H^2		0.64	0.50	0.61	0.66	0.42	0.70	0.62	0.83	0.75	0.51	0.77	0.37	0.90

Abbreviations: MR, moderately resistant; MRMS, moderately resistant to moderately susceptible; MS, moderately susceptible; R, resistant; S, susceptible.

^aOnly severity measurement recorded.

TABLE 4 Percent severity of stripe rust infection in six environments, including two locations and three years.

Genotype	OR18	OR19	OR21	CA18	CA19	CA21
Woody-1	0.0	0.0	0.0	3.0	0.0	0.0
Woody-2	0.0	0.0	0.0	1.0	0.0	0.0
DH140512	1.5	5.0	–	5.0	2.5	–
UC1322	0.0	3.5	0.0	20.0	2.5	0.0
Robust	33.2	83.3	29.4	–	20.0	35.7
Thoroughbred	36.4	82.5	52.6	–	50.0	72.5
Median	6.5	16.0	3.0	30.0	5.0	5.0
H^2	0.50	0.93	0.39	0.99	0.69	0.44

Note: When multiple ratings were taken in a given environment, only the final rating is presented here. Trial median severity and broad-sense heritability are also provided.

resistance genes in the Woodies, the overall rust phenotype could still be influenced by extreme temperatures. Subsequent reports have linked the timing of extreme temperature events and plant maturation date with the breakdown of resistance, corroborating reports of planting date as suitable management strategy (Mapuranga et al., 2022).

3.3 | Adult stripe rust resistance

Results from the adult plant stripe rust resistance trials are provided in Table 4. Stripe rust was only noted to affect the Woodies in one environment (CA18), with severity rates of 3.0% and 1.0% for Woody-1 and Woody-2, respectively. The parents, DH140512 and UC1322, had severity ranges of 1.5%–5.0% and 0.0%–20.0%, respectively. The susceptible check genotypes of ‘Robust’ (PI 476976) and ‘Thoroughbred’ (PI 634933) exhibited severities in the range of 20.0%–83.3%

and 50.0%–82.5%, respectively. A full table of stripe rust severity values for each genotype tested in each trial can be found in Supplemental Table S2, which also includes the standard error and within-environment grouping.

3.4 | Basis of resistance to stem and stripe rust

The genetic basis for the Woodies’ resistance to stem and stripe rust is proposed to be a QTL on chromosome 5H independent of the *rpg4/Rpg5* locus that is known to confer resistance to stem rust. Previous studies have investigated this aspect more in-depth. A full description of the methods used to identify this locus are described in Hernandez et al. (2020b). Briefly, a mapping population of 385 doubled haploid genotypes was screened for resistance to stem rust and stripe rust in four and three environments, respectively. A genome-wide association study was used to identify the 5H QTL, and haplotype analysis was used to identify a likely region of interest. The Woodies did not amplify for *Rpg5* in a polymerase chain reaction–based analysis.

3.5 | Agronomic characters

A summary of the agronomic assessment of the Woodies is provided in Table 5. Values of yield percentage for the woodies were at or slightly below the trial mean for each of the experimental locations. The Woodies tended to have lower plant height than the trial mean, with approximately average test weight and protein values. In the seed increase grown in Corvallis, OR, during the 2022 season, Woody-1 and Woody-2 reached heading at 172 and 173 days after January 1, respectively. Cultivars ‘Copeland’ (Gov. Canada Reg. 4991),

TABLE 5 Agronomic performance of Woody-1 and Woody-2 in the 2023 Oregon State Extension Spring Barley Variety Trials compared with the trial mean.

Location	Management	Genotype	YLD kg ha ⁻¹	PH cm	TW g L ⁻¹	Grain protein %
Athena	irrigated	Woody-1	5342	60.7	673	8.2
		Woody-2	4852	56.1	649	8.1
		trial mean	5658	64	672	8.3
Ione ^a	no-till	Woody-1	2598	58.7	606	16.3
		Woody-2	2885	57.4	614	16.8
		trial mean	2807	62.5	615	15.9
La Grande	irrigated	Woody-1	9044	68.6	685	11.3
		Woody-2	8534	64.8	686	11.7
		trial mean	9638	77	683	11.5
Pendleton	conventional tillage	Woody-1	1499	–	693	11.8
		Woody-2	1311	–	678	11.5
		trial mean	1883	–	696	11.4
Klamath Falls	irrigated	Woody-1	9030	72.4	712	13.3
		trial mean	8676	70.1	703	13.0

Abbreviations: PH, plant height; TW, test weight; YLD, grain yield.

^aDrought conditions at this location affected plant productivity.

TABLE 6 Malt quality values for the Woodies as determined by the Hartwick Center for Craft Food and Beverage following American Society of Brewing Chemists methods.

Genotype	Extract %	Color (°SRM)	β -glucan mg L ⁻¹	Protein %	S/T	FAN mg L ⁻¹	DP °L	α -amylase	>7/64 ^a %
Woody-1	83.2	2.84	96	10.5	57	287	157	66.8	67.5
Woody-2	82.7	2.44	64	12	49.6	285	165	73.4	72.8
Adjunct	>81	1.6–2.5	<100	≤12.8	40–47	>210	>140	>50	>70
All-Malt	>81	1.6–2.8	<100	≤11.8	38–45	140–190	110–150	40–70	>75

Note: Ideal values for both adjunct and all-malt brewing outlined by the American Malting Barley Association are also included.

Abbreviations: DP, diastatic power; FAN, free amino N; S/T, ratio of soluble to total protein.

Full Pint, and ‘Golden Promise’ (Foster, 2001) for reference reached heading at 180, 176, and 178 days after January 1, respectively.

3.6 | Malt quality

The malt quality assessment for the Woodies can be found in Table 6, along with the AMBA target values for selection of malting barley (AMBA, 2021). Based on these measurements, both of the Woodies meet the standard for either adjunct or all-malt in terms of their fine extract and β -glucan content. The total protein content of Woody-2 was more in line with adjunct brewing targets, whereas Woody-1 was acceptable for either class. In terms of color, Woody-1 was slightly high, and Woody-2 fell in the range for all-malt or adjunct brewing. Both

of the Woodies had slightly higher than desired soluble protein based on the ratio of soluble to total protein in this assessment. Free amino nitrogen, diastatic power, and α -amylase values for both of the Woodies were more aligned with an adjunct brewing barley.

4 | CONCLUSIONS

There is a need in the barley industry for disease-resistant cultivars and novel sources of resistance to stem rust and stripe rust. The two-row spring type genotypes Woody-1 and Woody-2 have displayed resistance to stem and stripe rust across a range of environmental conditions. The stripe rust tolerance was exceptional across all environments. Stem rust resistance did appear to be reduced in the 2022 season in

Washington when temperatures were high, an effect observed with some stem rust resistance genes. In terms of their malting quality, the Woodies were found to have acceptable character for either all-malt or adjunct brewing with respect to most traits, though with possibly more than desired soluble protein levels. Although their other agronomic characters are not groundbreaking, as carriers of a novel source of rust resistance, the Woodies are of great utility to a breeding program seeking new rust-resistant germplasm.

5 | AVAILABILITY

Seed is available on request from the Oregon State University Barley Breeding Program or from the National Laboratory for Genetic Resources Preservation Germplasm Repository. Data used in the creation of all tables and figures presented in this manuscript are available upon request. See <https://barleyworld.org/> for information regarding contacting the barley laboratory. The complete 50K SNP array genotype data for Woody-1 and Woody-2 are available at <https://barley.triticeatoolbox.org>.

AUTHOR CONTRIBUTIONS

C. Massman: Data curation; formal analysis; investigation; writing—original draft; writing—review and editing. **J. Hernandez:** Data curation; investigation; writing—review and editing. **S. J. Clare:** Writing—original draft; writing—review and editing. **M. Brooke:** Investigation; writing—review and editing. **T. Filichkin:** Resources; writing—review and editing. **S. Fisk:** Investigation; writing—review and editing. **L. Helgerson:** Investigation; writing—review and editing. **O. N. Matny:** Investigation; writing—review and editing. **I. A. del Blanco:** Investigation; writing—review and editing. **M. N. Rouse:** Investigation; writing—review and editing. **B. J. Steffenson:** Investigation; writing—review and editing. **B. Brueggeman:** Investigation; writing—review and editing. **P. M. Hayes:** Data curation; funding acquisition; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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