Powdery Mildew Resistant Hop Germplasm Release: ‘Kazak 2000’

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Abstract

A USDA-sponsored plant collecting expedition in collaboration with the Vavilov Institute of Plant Industry (VIR), St. Petersburg, Russian Federation, and the Aral Sea Experiment Station for Plant Genetic Resources, Chelkar Town, Kazakhstan, was conducted in the fall of 2000. Hop (*Humulus lupulus* var. *lupulus*) cones were collected from wild plants growing on trees in a moist area about 50 km NE of Emba, Kazakhstan. A portion of the seeds were brought to the US and donated to the USDA Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository in Corvallis, Oregon. Of 74 seedlings that were germinated from Kazakhstani seedlots, four seedlings from PI 635262 demonstrated resistance to powdery mildew, caused by *Podosphaera macularis* (Braun and Takamatsu). One of these resistant seedlings, ‘Kazak 2000’, exhibited a hypersensitive response after challenge by powdery mildew, especially when incubated at temperatures greater than 29°C post-infection. In greenhouse assays with an Oregon field population of *P. macularis*, macroscopic signs of powdery mildew were not observed following repeated inoculations. In laboratory assays at 18°C, ‘Kazak 2000’ did not develop powdery mildew when challenged with a *P. macularis* isolate capable of overcoming resistance genes *R*<sub>b</sub>, *R*<sub>3</sub>, and *R*<sub>s</sub>. Infection of 55% of detached leaves was observed when challenged with characterized isolates capable of overcoming *R*<sub>b</sub>, *R*<sub>3</sub>, *R*<sub>4</sub>, *R*<sub>5</sub>, *R*<sub>6</sub> or *R*<sub>b</sub>, *R*<sub>1</sub>, *R*<sub>2</sub>, *R*<sub>5</sub>, and *R*<sub>6</sub>, respectively. In experimental field plots in Oregon from 2003 to 2007, only one powdery mildew colony was observed despite inoculation and exposure to natural inoculum sources each year. Infection of cones was not observed. ‘Kazak 2000’ provides a novel source of powdery mildew resistance that is effective at high temperatures against multiple races of the pathogen. Limited quantities of cuttings of ‘Kazak 2000’ are available for research or breeding. Requests should be made to the Hop Curator, USDA-ARS, National Clonal Germplasm Repository, Corvallis, OR 97333.

INTRODUCTION

In 1997, the appearance of hop powdery mildew, caused by *Podosphaera macularis* Braun and Takamatsu (formerly *Sphaerotheca macularis* (Wallr.:Fr.) Lind syn. *S. humuli* (DC.) Burrill), in the Pacific Northwestern United States threatened commercial hop (*Humulus lupulus* L.) production due to the lack of management strategies or host resistance of the current commercial cultivars. Approximately 800 of 12,000 hectares in production were destroyed in 1997 due to hop powdery mildew (Ocamb et al., 1999). More than 1,200 hectares of highly susceptible hop cultivars were replaced, resulting in an estimated loss of $9.5 million in combined production and establishment costs (Turechek et al., 2001). Powdery mildew and its control is estimated to have cost Pacific Northwest hop growers over $30 million in 1999 and 2000, or about 15% of their total...
crop revenue (Turechek et al., 2001). Management of the disease is now accomplished largely through fungicide applications, which can exceed 10 applications per year at an estimated cost of approximately $1000/hectare (Gent et al., 2008).

The severity of the disease prompted American breeders and brewers to pursue development of cultivars resistant to powdery mildew. Collecting expeditions were conducted to seek wild germplasm to discover new sources of disease resistance. From 1999 to 2003, wild H. lupulus varieties were collected from North Dakota, Missouri, Colorado, New Mexico and Arizona and from Manitoba and Saskatchewan, Canada (Hampton et al., 2001; Hummer, 2005). In Fall 2000, Dr. Richard Hannan, Horticulturist from Pullman, Washington, lead a USDA-sponsored plant collecting expedition in collaboration with scientists at the Vavilov Institute of Plant Industry (VIR), St. Petersburg, Russian Federation, and the Aral Sea Experiment Station for Plant Genetic Resources, Chelkar Town, Kazakhstan (GRIN, 2008). During that expedition, hop (H. lupulus var. lupulus) cones were collected from wild plants growing on trees in a moist area about 50 km northeast of Emba.

About 2,000 North American and about 100 Kazakhstani seedlings were germinated from these recent collections and evaluated for powdery mildew resistance after artificial inoculation with an Oregon population of P. macularis. A seedling from Kazakhstan with powdery mildew resistance was identified. The objective of this project was to document the extent of powdery mildew resistance in this genotype, and release this clone as parental germplasm for future crosses and research.

MATERIALS AND METHODS

Initial Disease Screening

Conditions for seed germination included a 5 minute soak in a 10% sodium hypochlorite solution followed by a 10 minute rinse with distilled water. Depending on availability, 10, 25, 50 or 100 seeds from each lot were placed on moist, autoclaved sand in clear, plastic germination boxes. Germination boxes were held in the dark at 4°C for 6 weeks. After this pre-chilling period, the boxes were moved to a germination chamber where they received 14 hours of light at 26°C followed by 10 hours of dark at 10°C. As the radicles emerged, the seeds were removed and placed in cell packs of potting soil to continue germination under greenhouse conditions.

As the seedlings reached adequate size (approximately 15 cm in length), they were artificially inoculated with P. macularis (described below). A field population of P. macularis, consisting of several single-conidial chain isolates collected from of P. macularis obtained from hop yards in Oregon was used as inoculum. The isolates were maintained in a growth chamber through successive transfers on potted plants of H. lupulus ‘Symphony’ grown at 13°C with a 15h photoperiod.

Inoculum was prepared by collecting infected hop leaves from the growth chamber and washing the conidia from the leaves with a 0.005% (vol/vol) solution of Tween 20 and ultra-pure water (18 mΩ). Plants were inoculated with a spore suspension of \(2 \times 10^4\) conidia/ml until just before runoff using a hand-held atomizer (Nalge Nunc International, Rochester, NY). Leaves were air-dried within 1h of starting the preparation of the spore suspension to minimize lysis of conidia. Subsequent inoculations consisted of periodically mechanically shaking heavily infected leaves or whole plants over plants not showing infection to aid in the spread of conidia and minimize disease escapes.

Disease reactions of seedlings were categorized into 5 groups and rating using an ordinal scale where: 1 = no infection; 2 = a hypersensitive response (HR); 3 = one or a few small colonies with no active sporulation; 4 = moderate infection or medium sized colonies with moderate to high sporulation (reduced susceptibility); or 5 = high infection or many large colonies with high sporulation (susceptible). Plants in categories 1 or 2 were repotted into 3-liter pots and re-inoculated at least 5 times to verify disease reactions.
Field Observations

32 genotypes from categories 1 or 2 were selected for further evaluation under field conditions. The selections consisted of 29 native North American genotypes (27 *H. lupulus* var. *lupuloides* and 2 *H. lupulus* var. *pubescens*) and 3 native Kazakhstani (*H. lupulus* var. *lupulus*) genotypes. The selections were planted in a USDA-ARS experimental hop yard near Corvallis, Oregon in June 2003. Hop plants were slow to establish the first year, and field evaluations were taken beginning in 2004.

Plants were artificially inoculated with *P. macularis* each season to supplement natural sources of inoculum that were present in 2005, 2006, and 2007. Leaves and cones, when present, were inoculated 27 August in 2004, 9 August 2005, 8 August 2006, and 15 May 2007. Inoculum was prepared in the field and applied as described previously except that inoculum titer ranged from $1.5 \times 10^4$ to $2 \times 10^5$ conidia/ml. Plants were evaluated for leaf infections beginning one week after inoculation by inspecting 10 to 50 leaves per plant, depending on the number of leaves available, for signs of powdery mildew. At least two leaf evaluations were conducted each year. Near maturity, a subsample of 100 cones (where possible) were collected from female plants and evaluated for signs and symptoms of powdery mildew with the aid of a stereomicroscope. Disease incidence, i.e., the proportion of infected cones was determined based on the presence of signs of the pathogen.

Susceptibility to downy mildew, caused by *Pseudoperonospora humuli* (Miyabe & Takah.), was evaluated in 2006 and 2007. Natural outbreaks of downy mildew occurred annually, and provided inoculum to infect the plants. The number of systemically infected shoots (“spikes”) per plant was counted during three evaluations in each season.

Agronomic characteristics were measured during 2006 and 2007. Cone length was determined by measuring the length of 30 cones per plant each year. Yield was estimated for each plant by harvesting the entire plant at maturity and picking cones with a small-scale hop picking machine. Cones were dried to 8 to 12% moisture content before determining alpha and beta acid content by spectrophotometric methods.

Disease Screening with Typed Powdery Mildew Strains

There are genes known to confer resistance to hop powdery mildew in specific hop varieties and these seven “differential” hop varieties were used for screening powdery mildew to determine the virulence genes present in a mildew strain. Three isolates of *P. macularis* (Table 1) which have been typed for virulence on differential hop cultivars were used to evaluate powdery mildew resistance of ‘Kazak 2000’. Leaves were detached from the second node below the bine tip of 1-gal plants growing in a greenhouse. Detached leaves were immediately placed in Petri dish moist chambers (2-3 leaves per chamber) and returned to the laboratory. Using a hand-held needle, spores of *P. macularis* were inoculated on to leaves and only one powdery mildew strain was used in each moist chamber. Moist chambers containing the inoculated leaves were incubated at 18°C with 12-h day length, and examined under a stereoscope for powdery mildew growth. Mildew growth was recorded at 14 and 21 days after inoculation of leaves. For each screening, six leaves per hop variety were inoculated with one critical HPM isolate and all three critical HPM isolates were screened at each time. Each hop variety was screened three different years.

Infection during High Temperatures

Clonal plants of *H. lupulus* ‘Nugget’ (resistant), ‘Symphony’ (susceptible) and a wild Kazakhstani selection, ‘CHUM 1025.007’, (hypersensitive/tolerant) were produced from softwood cuttings, planted in 5-cm pots with Sunshine Mix (SunGro Horticulture, Bellevue, WA) and grown under greenhouse conditions with at least a 15-h photoperiod. Plants were fertilized with Osmocote (Scotts-Sierra Horticultural Products Co., Marysville, OH) slow-release fertilizer (14-14-14), watered as needed, and supplied with Champion 17-17-17 fertilizer with micronutrients (McConkey’s, Portland, OR) at each
watering. Plants were maintained powdery mildew free by vaporizing sulfur in the greenhouse each night for 4h.

Three one- to two-month-old, potted plants of each of three genotypes were grown for 10 days in growth chambers at 29, 32, and 35°C. To prevent contamination from airborne conidia, plants were grown inside plastic canisters with a 22 micron cloth lid to allow for air exchange. The canisters were set in trays filled with water to prevent conidial contamination from below. Temperature and relative humidity in the growth chambers were monitored with a HOBO pro Series RH/Temp data logger (Onset Computer Corp., Bourne, MA).

Using a hand-held atomizer, plants were inoculated as above, air dried, then placed into a growth chamber at 18°C (the optimal temperature for infection, growth, and sporulation of *P. macularis*) with a 15-h photoperiod. Non-inoculated plants were placed in the chamber as negative controls to determine background levels of infection.

After 10 days at 18°C, the infection frequency was determined for the adaxial surfaces of the four most apical, fully-expanded leaves at the time of inoculation. The infection frequency was determined by dividing the number of lesions per leaf by the leaf area (cm²). The infection frequency was then averaged across all leaves for each plant. Leaf area was determined by using the average of two measurements per leaf obtained with a Li-Cor LI-3000 leaf area meter (Li-Cor Inc., Lincoln, NE).

The experiment was a 3 × 3 factorial, arranged in a split-plot design with sub-sampling. The experiment was replicated three times with replication in time serving as the blocking factor. Temperature served as the whole-plot factor and genotype served as the sub-plot factor. The response variable was infection frequency, calculated as the total number of lesions on 12 leaves (4 leaves from each of 3 plants) divided by the total leaf area of the 12 leaves. Temperatures were randomly assigned to growth chambers for each replication. The assumptions of normality and homogeneity of variance were checked using the UNIVARIATE procedure in SAS (SAS Institute, Cary, NC) before analysis of variance using a general linear model in SAS.

RESULTS AND DISCUSSION

Initial Screening

Four seedlings from Kazakhstani seedlot PI 635262 demonstrated powdery mildew resistance. In six greenhouse assays with an Oregon field population of *P. macularis*, macroscopic signs of powdery mildew were not observed following repeated inoculations.

One of these resistant seedlings, (selection number ‘CHUM 1025.007’) exhibited a hypersensitive response after challenge by *P. macularis*. Within 96 hours of inoculation of the epidermal cells beneath the young powdery mildew colonies began to blister and became chlorotic. After 120 hours of incubation, fungal hyphae and conidiophores began to lyse and a minute necrotic lesion formed at the center of the pathogen colony and enlarged over time.

Infection during High Temperatures

When exposed to high temperatures prior to inoculation ‘Symphony’ exhibited a reduced susceptibility to *P. macularis* (Fig. 1), confirming the findings of Mahaffee et al. (2003). The pre-existing partial resistance of the Kazakhstani genotype was also increased by exposure to high temperatures prior to inoculation (Fig. 1.) No infection was found to occur on ‘Nugget’ at any temperature treatment (Fig. 1). These results imply that susceptible *Humulus* genotypes exhibit temperature dependent resistance.

Field Observations

‘Kazak 2000’ matures in early to mid-August in Oregon. Single plant cone yield estimates averaged 0.25 kg per hill in two years of field evaluation in Oregon. Average cone length was 2.9 cm. In 2006 and 2007, spectrophotometric analysis of alpha acid
content of cones ranged from 3.30 to 3.48% and beta acids range from 3.77 to 4.49%.
‘Kazak 2000’ is susceptible to downy mildew [caused by Pseudoperonospora humuli (Miyabe. & Takah.) G.W. Wilson], which is typical for many wild Humulus accessions (Haunold et al., 1993). In experimental plots in Oregon from 2003 to 2007, only one powdery mildew colony was observed on a leaf in 2007 despite artificial inoculation each year and exposure to natural sources of inoculum. Infection of cones was not observed during field evaluations.

**Screening with Typed Powdery Mildew Strains**

In laboratory assays at 18°C, ‘Kazak 2000’ did not develop powdery mildew when challenged with isolate C100, a P. macularis isolate capable of overcoming resistance genes Rb, R3, and R5 (Table 1). When challenged with isolate C104, capable of overcoming Rb, R3, R4, R5, R6, 28% of the leaves inoculated developed powdery mildew and colony size at 14 days averaged 2.25 mm in diameter. Infection of 55% of detached leaves was observed when leaves were inoculated with isolate C107 and colony diameter averaged 1.54 mm.

**CONCLUSIONS**

The release of selection ‘CHUM 1025.007’ as ‘Kazak 2000’ provides a novel source of partial powdery mildew resistance that is effective at high temperatures (Fig. 1). ‘Kazak 2000,’ and the original Kazakhstani seedlot from which it was selected, were deposited in the hop genebank in Corvallis, Oregon. Limited quantities of cuttings of ‘Kazak 2000’ are available for research or breeding. Requests should be made to the Hop Curator, USDA-ARS, National Clonal Germplasm Repository, Corvallis, OR 97333, or through the USDA ARS, Germplasm Resources Information Network (GRIN) at http://www.ars-grin.gov/npgs/order.html. Additional background information on this clone can be viewed on the GRIN database: http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1612427.

**ACKNOWLEDGEMENTS**

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**Literature Cited**


Tables

Table 1. Differential hop cultivars and associated resistance genes.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Genes that confer resistance to <em>P. macularis</em></th>
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<tr>
<td></td>
<td>Rb</td>
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<tr>
<td>Yeoman</td>
<td>BN+</td>
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<tr>
<td>Zenith</td>
<td>BN+</td>
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<tr>
<td>Wye Target</td>
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<td>Wye Challenger</td>
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<td>Serebrianka</td>
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<td>Early Choice</td>
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<td>Nugget</td>
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Table 2. Virulence of isolates used to characterize powdery mildew resistance in *Humulus lupulus* accession ‘Kazak 2000’ (‘CHUM 1025.007’).

<table>
<thead>
<tr>
<th><em>P. macularis</em> isolate</th>
<th>Yeoman (R_b)</th>
<th>Zenith (R_b, R_1, R_3)</th>
<th>Wye Target (R_2)</th>
<th>Wye Challenger (R_b, R_3)</th>
<th>Serebrianka (R_4)</th>
<th>Early Choice (R_5)</th>
<th>Nugget (R_6)</th>
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<tbody>
<tr>
<td>C100</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C104</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>C107</td>
<td>+</td>
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<td>+</td>
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*a* *P. macularis* isolates C100, C104, and C107 were obtained in 2004 from cultivars ‘Nugget’, ‘Chinook’ and ‘Galena’, respectively.

+ = Powdery mildew colony grew on detached leaves.

- = No colony growth.
Fig. 1. Mean infection frequency of powdery mildew on the resistant genotype, *Humulus lupulus* ‘Nugget’ (= Nug), ‘Kazak 2000’ (= Kaz), and the susceptible genotype ‘Symphony’ (= Sym) after exposure to pre-inoculation temperatures of 29, 32, or 35°C for 10 days. Disease was assessed microscopically 10 days after inoculation with a field population of *Podosphaera macularis* collected in Oregon and subsequent incubation at 18°C.