

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

in cooperation with

STATE AGRICULTURAL EXPERIMENT STATIONS

Results from the

UNIFORM OATS WINTER HARDINESS NURSERY

2008-2009

Compiled by

D. P. Livingston, Research Agronomist
T. D. Tuong, Plant Science Research Technician
Jeanette Lyerly and Peter Maloney

This is a joint progress report of an investigation underway in the State Agricultural Experiment Stations and the Agricultural Research Service of the U. S. Department of Agriculture. It contains preliminary data which have not been sufficiently confirmed to justify general release; interpretations may be modified with additional experimentation. Confirmed results will be published through established channels. The report is primarily a tool for cooperators, their staff and those with special interest in agricultural research program development.

This report was compiled by the Agricultural Research Service, U. S. Department of Agriculture, and is not intended for publication nor should it be referred to in literature citations or quoted in publicity or advertising. Use of the data may be granted for certain purposes upon written request to the agency or agencies involved.

USDA-ARS
South Atlantic Area
Department of Crop Science
North Carolina State University
Raleigh, NC 27695

CONTENTS

Cooperating Agricultural Experiment Stations and Personnel	Page 2
Digest and Comments	3
Table 1. Entries in the 2008-2009 Uniform Oat Winter Hardiness Nursery	4
Top Ten Ranked Survival Entries	5
Table 2. Uniform Oat Winter Hardiness Nursery Control Tests	6
Table 3a. Percent Survival at the Various Stations (sorted by entry no.)	7
Table 3b. Percent Survival at the Various Stations (sorted by rank)	7
Introduction to Marker-Assisted Selection	8
SSR Analyses with Markers Associated with Winter Hardiness Traits	9
Table 4. SSR Analyses with Markers	10,11

COOPERATING AGRICULTURAL EXPERIMENT STATIONS AND PERSONNEL

Country	State	AES Location	Personnel
USA	AR	Fayetteville	J. Kelly
USA	IL	Lawrenceville	L. Phillippe
USA	SC	Clemson	B. Edge
USA	NC	Laurel Springs/Waynesville	D. Marshall/M. Fountain
USA	TN	Knoxville	D. West
USA	LA	Baton Rouge	S. Harrison
Turkey	K.Maras	KSU Field Crops Department	T. Dokuyucu
Lithuania	Akademija	Lithuanian Univ. of Agriculture	A. Sliesaravicius
Poland	Blonie	Plant Breeding and Acclimatization Ins.	B. Lapinski
Czech Republic	Kromeriz	Agricultural Research Institute	L. Nedomova
Hungary	Martonvasar	Agric. Res. Inst. of Hungary Academy	O. Veisz
UK	Aberystwyth	IGER Plas Gogerddan	S. Cowan
Austria	Edelhof	Saatzucht Edelhof	S. Berger

DIGEST

NUMBER OF TESTS:	13 tests (6 US States, 7 foreign countries)	
NUMBER OF ENTRIES:	13	
EXPERIMENTAL DESIGN:	Single-row, 5-foot plot Two replications Randomized complete block	
DATA RECORDED:	Percent winter survival	
DATA NOT USED IN ANALYSIS:	Fayetteville, AR	100% Survival
	Baton Rouge, LA	100% Survival
	Zwettl, Austria	0% Survival
	Clemson, SC	Data not received
	Wales, UK	Not planted due to wet conditions

COMMENTS:

- Analysis of markers associated with winter hardiness is implemented beginning with the 2008-2009 nursery.

US STATE/COUNTRY	LOCATION	COOPERATORS' COMMENTS
Poland	Radzików	Winter was mild with cold nights causing differentiated cold damages. Snow cover was not uniform resulting in inconsistent results between reps.
Czech Republic	Kromeriz	Plants were damaged by drought in April. Average temperature was 4.6°C with only 8% precipitation.
Lithuania	Akademija	Cold winter with temperature 20°C below zero.
Hungary	Martonvasar	Mild winter, dry spring.
Austria	Edelhof	No survivors! Late sowing (October 2008) due to arrival of seeds. First freeze was on 10-18-08. Very low temperatures in January. Long time closed snow layer until mid March.
Turkey	Afsin	Afsin's altitude is 1200 metres.

Table 1. Entries in the 2008-2009 Uniform Oat Winter Hardiness Nursery.

Entry No	Entry name	Pedigree	Yrs in Nursery	Contributors	
1	Fulgum (ck)	CI 708	71		
2	Norline (ck)	CI 6903	48		
3	Winter Turf (ck)	CI 3296	68		
4	Wintok (ck)	CI 3424	68		
5	NC03-2567v	H441/NC88-1652//NC88-1652	1	Murphy	NC
6	NC05-5460y	NC95-6073//TAM397/NC93-5856	1	Murphy	NC
7	ACS833	833 - released variety	1	Harrison	LA
8	Horizon 270	H270 - released variety	1	Harrison	LA
9	LA604	LA604 - released variety	1	Harrison	LA
10	LA99017SBSBSB-275-C	TX96M1385/LA604	1	Harrison	LA
11	Win/Nor-1	Wintok x Norline	6	Livingston, Murphy	NC
12	Win/Nor-10	Wintok x Norline	6	Livingston, Murphy	NC
13	Win/Nor-10b	Selection from Win/Nor-10	5	Livingston, Murphy	NC

Top Ten Ranked Survival Entries

Top 10 ranked survival entries for 2008-2009

Rank	Ent No.	Entry	Pedigree	% Survival (across locations)
1	10	LA99017SBSBSB-275-C-B-S1	TX96M1385/LA604	74.4
2	11	Win/Nor-1	Wintok x Norline	63.3
3	6	NC05-5460y	NC95-6073//TAM397/NC93-5856	63.2
4	4	Wintok (ck)	CI 3424	62.5
5	2	Norline (ck)	CI 6903	62.0
6	13	Win/Nor-10b	Selection from Win/Nor-10	61.0
7	12	Win/Nor-10	Wintok x Norline	60.6
8	9	LA604	LA604 - released variety	58.0
9	7	ACS833	833 - released variety	52.9
10	8	Horizon 270	H270 - released variety	50.7
LSD (0.05)				15.6

**Table 2. Uniform Oats Winter Hardiness Nursery
Under Controlled Environment Freeze Test**

Entry #	Entry Name	Survival Rating ¹	% Survival ²
1	Fulgum (ck)	0.2	10
2	Norline (ck)	2.3	83
3	Winter Turf (ck)	1.7	60
4	Wintok (ck)	3.0	90
5	NC03-2567v	1.8	53
6	NC05-5460y	1.4	58
7	ACS833	1.2	53
8	Horizon 270	0.7	35
9	LA604	2.0	70
10	LA99017SBSBSB-275-C-B-S1	2.6	85
11	Win/Nor-1	2.7	88
12	Win/Nor-10	2.9	95
13	Win/Nor-10b	2.9	98
Average		1.9	67
LSD (5%)		0.6	18
CV		14.4	12

Parameters:

- 2 reps/10 plants per rep planted in cone-tainers (Livingston et al. 2005, Crop Science, 45:1545-1558)
- 5 weeks at 13°C; 12 hours light/dark period; 400µmole light intensity
- 3 weeks at 3°C; 12 hours light/dark period; 350µmole light intensity
- 3 days @ -3°C in the dark (subzero acclimation)
- Frozen @ 1°C/hour to -12°C for 3 hours
- Thawed @ 2°C/hour to 3°C
- Plants were watered once with 0.001% (v/v) Vitavax fungicide solution
- Plants were allowed to recover for 3 weeks at 13°C in cone-tainers; 12 hours light/dark period; 400µmole light
- Plant survival ratings were rated for regrowth after 21 days by visually assessing leaves and roots.

¹Rating:

- 0** = Completely dead
- 1** = 1 survived (green) shoot or 1 primary root
- 2** = 1 or 2 survived (green) shoots or 1 survived shoot and 1 or 2 primary roots
- 3** = 1 or 2 survived shoots with developed roots (primary and secondary roots)
- 4** = 95% survived shoots with well developed roots
- 5** = 100% survived with very little or no sign of freeze damage

²Survival (%):

- 50% of plants with rating of 1plus all plants rated >2 divided by total number of plants frozen multiplied by 100

Table 3a. Winter Oat Survival (%) at Various Stations (sorted by entry number)

Ent. No.	Entry Name	Ranked Means	Means across loc	Laurel Springs NC	Waynesville NC	Afsin Turkey	Knoxville TN	Radzikow Poland	Kromeriz Czech Republic	Martonvasar Hungary	Lawrenceville IL	Akademija Lithuania
1	Fulgum (ck)	13	39.3	0	53	47	93	39	2	55	66	0
2	Norline (ck)	5	62.0	35	95	72	100	89	12	80	75	0
3	Winter Turf (ck)	11	49.9	1	78	63	100	73	15	70	50	0
4	Wintok (ck)	4	62.5	88	100	49	100	60	40	86	40	0
5	NC03-2567v	12	48.2	0	83	63	100	61	19	89	20	0
6	NC05-5460y	3	63.2	33	100	44	100	81	39	85	48	40
7	ACS833	9	52.9	23	100	74	100	65	16	77	10	13
8	Horizon 270	10	50.7	3	83	60	100	79	32	80	20	0
9	LA604	8	58.0	43	98	42	100	87	46	76	10	22
10	LA99017SBSBSB-275-C-B-S1	1	74.4	55	93	47	100	96	47	92	60	80
11	Win/Nor-1	2	63.3	-	-	51	100	100	54	99	40	0
12	Win/Nor-10	7	60.6	-	-	50	100	100	37	91	20	27
13	Win/Nor-10b	6	61.0	-	-	55	100	100	30	95	0	48
Average			57.4	27.9	88.0	55.2	99.4	79.3	29.8	82.4	35.2	17.5
LSD (0.05)			15.6	*ns	17.4	*ns	2.1	*ns	16.6	20.8	*ns	20.8
CV(%)			12.5	93.7	8.7	29.5	0.9	27.8	25.5	11.6	74.8	54.5

Table 3b. Winter Oat Survival (%) at Various Stations (sorted by rank)

Ent. No.	Entry Name	Ranked Means	Means across loc	Laurel Springs NC	Waynesville NC	Afsin Turkey	Knoxville TN	Radzikow Poland	Kromeriz Czech Republic	Martonvasar Hungary	Lawrenceville IL	Akademija Lithuania
10	LA99017SBSBSB-275-C-B-S1	1	74.4	55	93	47	100	96	47	92	60	80
11	Win/Nor-1	2	63.3	-	-	51	100	100	54	99	40	0
6	NC05-5460y	3	63.2	33	100	44	100	81	39	85	48	40
4	Wintok (ck)	4	62.5	88	100	49	100	60	40	86	40	0
2	Norline (ck)	5	62.0	35	95	72	100	89	12	80	75	0
13	Win/Nor-10b	6	61.0	-	-	55	100	100	30	95	0	48
12	Win/Nor-10	7	60.6	-	-	50	100	100	37	91	20	27
9	LA604	8	58.0	43	98	42	100	87	46	76	10	22
7	ACS833	9	52.9	23	100	74	100	65	16	77	10	13
8	Horizon 270	10	50.7	3	83	60	100	79	32	80	20	0
3	Winter Turf (ck)	11	49.9	1	78	63	100	73	15	70	50	0
5	NC03-2567v	12	48.2	0	83	63	100	61	19	89	20	0
1	Fulgum (ck)	13	39.3	0	53	47	93	39	2	55	66	0
Average			57.4	39.7	95.4	55.2	99.4	79.3	29.8	82.4	35.2	17.5
LSD (0.05)			15.6	*ns	17.4	*ns	2.1	*ns	16.6	20.8	*ns	20.8
CV(%)			12.5	93.7	8.7	29.5	0.9	27.8	25.5	11.6	74.8	54.5

*ns = not significant

Introduction to Marker-Assisted Selection (MAS)

Traditional phenotypic-pedigree-based systems have been very successful in improving desired traits in plants. However, these systems can be time consuming. Advancements in genetic technology have allowed for development of a system that can shorten the selection process using the results of DNA testing known as Marker-Assisted Selection (MAS). Breeders can utilize data generated from MAS to “assist” or use in conjunction with observations from field tests to develop a variety in a shorter time period by enabling selection of a desired trait, such as winter hardiness, in an earlier generation.

Winter hardiness is related to multiple quantitative traits, including winter field survival, crown freezing tolerance, vernalization response, and photoperiod. Crown freezing tolerance (CFT) is measured in controlled freeze tests and is an important component to winter hardiness. Improved CFT, root scores (RS) and leaf scores (LS) indicate better recovery in controlled freeze tests. The 7C-17 translocation (TR) is thought to contain a cluster of genes for increased tolerance to freezing temperatures and has been significantly correlated with winter field survival and crown freezing tolerance. Photoperiod (PPD) and vernalization response (VRN) are frequently correlated with winter field survival and freezing tolerance because these traits contribute to delaying new growth until after the danger of freezing temperatures has passed. This avoidance response, affected by the combination of certain photoperiod and vernalization traits, can be useful for increasing winter field survival.

Genetic markers are fragments of DNA that are linked with known genes or traits. Associating markers with winter hardiness component traits (above) provides a valuable tool for oat research programs. Simple sequence repeats, known as SSRs or microsatellites, are a popular marker choice due to their relative low cost and ease of use. Oat SSR markers were previously evaluated for their association with winter hardiness component traits in a ‘Fulghum’ x ‘Norline’ population. The SSR markers associated with selected traits were chosen for testing with lines from the Uniform Oats Winter Hardiness Nursery, and the data are presented in Table 4.

If the DNA fragment associated with the phenotype of interest is present (associated fragments are shown in bold), this suggests that the line may have the winter hardiness trait. For example, the SSR marker HVM20 is associated with leaf score, root score, crown freeze tolerance, and the 7C-17 translocation. HVM20 produces two fragments with sizes of 132 base pairs (bp) and 142bp (table 4, page 10). The presence of the 142bp fragment could translate to increased crown freeze tolerance, improved root scores, and improved leaf scores, and would be desirable in a marker-assisted selection program. In contrast, the SSR Primer 3 (table 4, page 11) was not informative for the lines tested in the nursery, even though the marker is associated with winter hardiness component traits.

In the case of winter hardiness, a combination of traits is necessary, and marker selection at this stage is preliminary. Even though entries in the nursery may be winter hardy, they may not necessarily possess all the winter hardiness component markers. Further research will clarify which combination of traits, and therefore which SSR markers, are most informative for the development of a marker-assisted selection program.

SSR Analyses with Markers Associated with Winter Hardiness Traits

Entries in the 2008-2009 Oat Winter Hardiness Nursery were evaluated with SSR markers associated with winter hardiness traits identified by Maloney et al. (unpublished). Marker data are given in base pairs (bp), where all forward primers have the M13(-19) sequence added for universal fluorescent labeling. Alleles associated with positive effects on winter hardiness traits in the ‘Fulghum’ x ‘Norline’ mapping population are indicated in **bold**. Alleles that were not polymorphic for any lines are not reported.

1. Trait abbreviations and descriptions are as follows:

WFS = Winter field survival. Winter field survival was estimated after spring re-growth as the percent survival for the plots corrected for plot variation in germination or fall growth.

TR = Presence or absence of the translocation 7C-17. Fulghum non-T7C-17, Norline T7C-17. Presence of the translocation is associated with increased crown freezing tolerance (Wooten et al. *Crop Sci* 47:1832-1840 (2007)).

PPD = Photoperiod. Includes long and short photoperiod effects after 42 days of differing photoperiod treatments. Photoperiod, combined with vernalization and heading date, can mediate avoidance of freezing temperatures

VRN = Vernalization. Vernalization response to differing temperature treatments during seed germination, as described in Wooten et al. (*Crop Sci* (2009) in press). Vernalization time, along with photoperiod and heading date, may result in avoidance of freezing temperatures.

CFT = Crown Freeze Tolerance. Crown freezing tolerance was evaluated by preparing and freezing crowns in a controlled environment chamber as described by Wooten et al. (2009). After three weeks of re-growth, recovery for each crown was visually measured on a scale of 0-10 (0=complete plant death, 10=no freezing damage). LS = Leaf score. The freezing damage to the leaves only on a 1-5 scale. RS = Root Score. The freezing damage to roots only on a 1-5 scale.

2. JAO primer sequences may be requested from Joe Anderson at USDA-ARS, Purdue University.
3. VRN1 fragments are amplified by PoidVRN1 and Asintron-Rev in: Preston, J.C. and Kellogg, E.A. (2008), *Plant Physiol.* 146, no. 1: 265-276. VRN1 is involved in regulating response to vernalization in wheat and oats.
4. Sequences for Primer 1 through Primer 3 have not yet been released; therefore primer names have been coded. All other primer sequences, unless otherwise noted, are available in GrainGenes.

Table 4. SSR Analyses with Markers Associated with Winter Hardiness Traits (continue on next page)

	SSR Primer	AM2	AM102	AM270S-1	HVM20	JAO4042 ²	JAO4234
	Traits ¹	RS, LS, CFT	RS, LS, CFT	TR, LS, CFT	LS, RS, CFT, TR	TR	VRN, RS, LS, CFT
Entry No.	Entry name	Fragment size (# of base pairs)					
1	Fulghum (ck)	165	236	null	132	278, 272	253
2	Norline (ck)	157	221	206	142	278, 262	283 , 143
3	WinterTurf (ck)	165	221	null	132	278, 262	235
4	Wintok (ck)	165	221	206	142	278, 262	283
5	NC03-2567V	157	221	206	142	283, 262	249
6	NC05-5460Y	161	221	206	142	278, 262	249
7	ACS833	171, 165	221	206	142	278, 262	283 , 253
8	HORIZON 270	159	221	null	142	278, 262	253
9	LA604	171	221	206	142	283, 262	283
10	LA99017SBSBSB-275-C-B-S1	159	221	206	142	283, 262	247
11	WIN/NOR-1	165	221	206	142	278, 262	283
12	WIN-NOR-10	165	221	206	142	278, 262	283
13	WIN-NOR-10B	165	221	206	142	278, 262	283

WFS = Winter field survival.

TR = Presence or absence of the translocation 7C-17P

PD = Photoperiod.

VRN = Vernalization.

CFT = Crown Freeze Tolerance

LS = Leaf score

RS = Root Score

CFT = Crown Freeze Tolerance

Table 4(cont.) SSR Analyses with Markers Associated with Winter Hardiness Traits

	SSR Primer	VRN1 ³	Primer 1 ⁴	Primer 2	Primer 3
	Traits ¹	VRN	PPD, VRN, RS, LS, CFT	TR	WFS, PPD, VRN
Entry No.	Entry name	Fragment size (# of base pairs)			
1	Fulghum (ck)	960	235	null	193, 190
2	Norline (ck)	390	265	224	null
3	WinterTurf (ck)	960	265	224	193
4	Wintok (ck)	390	265	null	193
5	NC03-2567V	960	258	null	null
6	NC05-5460Y	960	256	224	193
7	ACS833	960	265, 256	224	193
8	HORIZON 270	960	256	224	193
9	LA604	960	265, 253	224	null
10	LA99017SBSBSB-275-C-B-S1	960	258	224	193
11	WIN/NOR-1	390	265	224	193
12	WIN-NOR-10	390	265	224	193
13	WIN-NOR-10B	390	265	224	null

WFS = Winter field survival.

TR = Presence or absence of the translocation 7C-17P

PD = Photoperiod.

VRN = Vernalization.

CFT = Crown Freeze Tolerance

LS = Leaf score

RS = Root Score

CFT = Crown Freeze Tolerance