Registration of Four Winter-Hardy Faba Bean Germplasm Lines for Use in Winter Pulse and Cover Crop Development

Erik J. Landry, Jolene E. Lafferty, Clarice J. Coyne, William L. Pan, and Jinguo Hu*

Abstract

Faba bean (Vicia faba L.) is one of the oldest domesticated crops and is widely grown as food, feed, vegetable, and forage (Rubiales, 2010). The world production of faba bean was 3.4 million metric tons in 2013 (FAOSTAT, 2014). The importance of faba bean as a specialty crop in the United States, especially for certain immigrant and ethnic groups, is augmented by its dual-purpose use as a cover crop or green manure. Faba bean has been noted for its high biological nitrogen fixation capacity (Herridge et al., 1994; Jensen et al., 2010) and biomass production (Hickman and Canevari, 2012) among the major cool-season food legumes. In areas where winter rains exacerbate soil compaction and erosion, faba bean as a winter cover crop can provide vegetative soil cover, increasing soil porosity and minimizing runoff and soil erosion. Faba bean is also an excellent host for insect pollinators when in flower from March to July in the Pacific Northwest conditions (Bugg et al., 1989).

The majority of faba bean germplasm is tolerant of cool conditions and light frost through the seedling stage but are not necessarily winter-hardy. True winter-type faba bean was reportedly introduced to England during the early 19th century, possibly via Russia (Lawes et al., 1983; Bond and Crofton, 1999). True winter-type faba bean germplasm from northern Europe is generally small seeded (<80 g 100 seed−1) and shows a vernalization requirement for early-node flowering, in contrast to spring types (Saxena, 1982; Ney and Duc, 1997; Link and Bond, 2011). A protracted rosette appears to be associated with an increase in freezing tolerance (less than −10°C) (Herzog 1988; Arbaoui et al., 2008). ‘Côte d’Or’ is reportedly the ultimate source of winter hardiness, with 61% survival after an extreme low of −25°C (Picard et al., 1985). We report here the development of faba bean germplasm tolerant to −25°C after six cycles of bulk selection for overwintering in Central Ferry and Pullman, WA: WH-1 (Reg. No. GP-3, PI 674326), WH-2 (Reg. No. GP-4, PI 674327), WH-3 (Reg. No. GP-5, PI 674328), and WH-4 (Reg. No. GP-6, PI 674329).

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Abbreviations: CF, Central Ferry farm; SF, Spillman agronomy farm; WF, Whitlow farm.
Materials and Methods

Bulk selection for overwintering was practiced over six seasons, from screening germplasm in 2008–2009 to validation in 2013–2014 (Table 1). No effort was made to prevent outcrossing between populations and among plants within populations, and mature seeds were sampled from bulk-harvested plots for the next cycle of selection. Two selection locations in southeastern Washington were used based on contrasting mild (Central Ferry Farm [CF]), Central Ferry, WA; 46°43′52″ N, 117°39′52″ W; elevation: 198 m) and severe (Whitlow Farm [WF], Pullman, WA; 46°43′28″ N, 117°08′07″ W; elevation: 790 m) winter selection pressures. Seed for each cycle of selection was sourced from WF, except in 2009–2010 when there was a complete crop loss (Table 1) and seed from CF was used, where survival averaged 5% across entries (Mwengi, 2011).

In October 2008, 43 faba bean accessions (USDA-ARS, Western Regional Plant Introduction Station, Pullman, WA) from seven countries, along with three commercial and 12 European winter-hardy cultivars and breeding lines, were autumn sown to screen for winter hardiness (Hu et al., 2009). In 2009, an additional 132 accessions were screened along with winter-hardy materials from the previous winter. These experiments identified several PI accessions that had winter-hardiness scores comparable to winter-type European cultivars (Mwengi, 2011). Four accessions with temporary accession ID numbers W6 12023, W6 12024, W6 12025, and W6 12028 and a European winter-hardy check Wibo/1† (obtained as an inbred line from W. Link) were among those chosen for further bulk selection. Passport data indicated that W6 12023, W6 12024, W6 12025, and W6 12028 were received from the K. Malkov Agricultural Experiment Station of the Institute of Introduction and Plant Genetic Resources Center in Sadovo, Plovdiv, Bulgaria, in 1987 as 347-2, A1, A4, and ’Webo’, respectively. Webo is a German cultivar registered in 1979 (Herzog, 1988), and the remaining W6 accessions are likely closely related (Kwon et al., 2010), but their definitive origin remains unknown.

The experimental design for the 2009–2010 and 2010–2011 seasons was slightly different from 2008–2009. Three replications of single-row plots 4.6 m long were seeded at 10 seeds m⁻¹ in 2009 on 15 and 16 September for WF and CF, respectively. The seeding dates were 22 September for WF and 8 October for CF in 2010. Slight alterations were made for the 2011–2014 seasons, and an additional field site in Pullman, WA, was included (Spillman Agronomy Farm [SF], 46°41′45.1″ N, 117°9′2.7″ W; elevation: 770 m). Plot dimension varied by location (1.5 × 1.8 m at WF and SF and 0.75 × 3.6 m at CF), but area was consistent (2.7 m²). Plots comprised four rows at WF and SF, but two at CF. Rows were spaced 35 cm apart at each location, with a 40-cm space separating plots. A Hege 120 planter was set for 48 seeds per plot.


Percentage survival (Table 1), flowering, 100 seed weight, mature height, mature branch number, and per plant yield (Table 2) are presented for the 2013–2014 autumn-sown trial. Whole plots were scored for percentage survival and flowering on 16 May at SF and WF. Height (cm), branch number, per plant yield (g plant⁻¹), and 100 seed weight was determined from five representative plants per plot at harvest.

A spring sowing (24 Apr. 2014) at WF using both original and selected populations was instituted to allow comparisons with the autumn-sown trial. The experimental design was a RCBD with four replications. Fifteen seeds were sown equidistant per single 1.5-m row plot and thinned to nine seedlings. Rows were spaced 35 cm apart, and plots were caged at flowering to eliminate outcrossing. Two seedlings on each end of the row served as borders, and the five remaining plants were averaged for height (cm), 100 seed weight, and per plant yield (g plant⁻¹) at harvest (5 September).

An analysis of variance was constructed separately for the 2013–2014 winter and spring trials using PROC MIXED of SAS 9.2 (SAS Institute, Cary, NC), and significance was set at P < 0.05 using Tukey’s honest significant difference test for means separation. Population and location effects were treated as fixed effects and block was set as a random effect within the model following a RCBD.

Table 1. Percentage survival of faba bean and lowest recorded temperature overwinter at Whitlow Farm, Pullman, WA, 2008–2014. Mean percentage survival across Whitlow and Spillman Agronomy Farms, Pullman, WA, is presented for the 2013–2014 season. Unselected entries for the 2013–2014 season were germplasm stocks of their representative W6 accessions, and 2009–2010 seed sourced from the Central Ferry Farm site in Central Ferry, WA, was used as unselected Wibo/1.

<table>
<thead>
<tr>
<th>Season</th>
<th>WH-1</th>
<th>WH-2</th>
<th>WH-3</th>
<th>WH-4</th>
<th>Wibo/1†</th>
<th>Lowest air temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008–2009</td>
<td>50</td>
<td>83</td>
<td>62</td>
<td>76</td>
<td>NA†</td>
<td>−22.8</td>
</tr>
<tr>
<td>2009–2010</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>−18.9</td>
</tr>
<tr>
<td>2010–2011</td>
<td>37</td>
<td>37</td>
<td>70</td>
<td>77</td>
<td>67</td>
<td>−15.4</td>
</tr>
<tr>
<td>2011–2012</td>
<td>68</td>
<td>61</td>
<td>64</td>
<td>61</td>
<td>73</td>
<td>−11.7</td>
</tr>
<tr>
<td>2012–2013</td>
<td>53</td>
<td>75</td>
<td>65</td>
<td>63</td>
<td>71</td>
<td>−14.5</td>
</tr>
<tr>
<td>2013–2014 (selected)</td>
<td>32</td>
<td>39</td>
<td>43</td>
<td>37</td>
<td>47</td>
<td>−24.7</td>
</tr>
</tbody>
</table>

† Original seed sourced from the breeding program of Dr. Wolfgang Link, Georg-August-University.
‡ NA, not available.
**Characteristics**

The winter hardiness of four germplasm lines was enhanced after six consecutive cycles of natural selection by an average of 84% (Table 1). We selected winter-hardy 1 (WH-1), winter-hardy 2 (WH-2), winter-hardy 3 (WH-3), and winter-hardy 4 (WH-4) bulk populations from W6 12023, W6 12024, W6 12025, and W6 12028, respectively. The response to bulk selection of WH lines in comparison to original W6 accessions is discussed in relation to Wibo/1.

Mean branch number (1.6–2.1) and height (56.9–62.1 cm) at maturity and per plant yield (26.6–38.6 g) of selected populations across WF and SF in 2013–2014 were similar (Table 2). In our experience, yield is affected by winter injury, seasonal soil moisture, and temperature fluctuation during flowering. For example, after the mild winter of 2011–2012, the branch number and height at maturity and plant yield of these populations averaged across WF, SF, and CF was >3.5, 90 cm and 50 g, respectively. Conditions that limit mortality and support growth overwinter, such as at CF in 2011–2012, maximize yield potential, with plot yields from 6.9 to 8.5 t ha⁻¹ depending on population.

These winter faba bean populations appear to be facultative winter annuals. The spring sowing in 2014 on average taller at maturity (94.4 cm) and higher yielding (68 g) than the autumn-sown trial of 2013–2014. In general, mature height and branching tend to be greater when winter-type populations are spring-sown, possibly as a result of avoiding winter injury (Landry et al., 2015). However, yield of spring-sown plants are typically low (<20 g plant⁻¹), due to later flowering (June–July vs. May–June) and subsequent heat and drought stress. Greater height and per plant yield of the spring sowing in 2014 may have been the result of caging at flower initiation in an effort to eliminate outcrossing. Lower light intensity and temperature may have stimulated internode elongation and pod set, respectively. Interestingly, the mean per plant yield for the WH populations (99 g plant⁻¹) was substantially higher than the original selections (37 g plant⁻¹) on average, but no appreciable change was observed between selected and unselected populations for branching or height at maturity.

**Discussion**

The successful survival of overwintered faba bean grown in southeastern Washington is highly influenced by environmental fluctuations from year to year (Table 1). The crop failure of 2009–2010 was likely the result of air temperature lower than −10°C during germination in October and minimal snow cover and a soil temperature of −7.5°C at a 15 cm depth (Saxena, 1982; Mwengi, 2011). From 2010 to 2013 the winters were relatively mild. The 2013–2014 winter, however, had limited snow cover and was cold enough to distinguish differences between selected and unselected populations.

Complicating the influence of prevailing environmental conditions, planting date can also affect overwintering. Late sowings, as was the case at CF in 2013–2014, resulted in crop failure because of inadequate hardening and a −75°C temperature at seed depth during germination. A general recommendation for much of southeastern Washington is to sow by the first week of October. Generally, this coincides with daily average air (<8°C) and soil (<10°C) temperatures (Murray et al., 1988), but early hard freezes can seriously compromise stand establishment.

There was no location × population interaction for percentage survival in 2013–2014, but there was a significant population effect, so percentage survival for each population was averaged across the SF and WF locations. In general, the WH populations were harder than their original W6 accessions; however, none surpassed the hardiness of selected Wibo/1 (Table 1).

Flowering was one morphological trait that appeared to shift for at least W6 12023, from early flowering in the unselected accession, to a range similar to the other populations after selection (Table 2). Furthermore, for W6 12023, 100 seed weight was reduced 14%, whereas the others remained relatively unaltered, except for Wibo/1, which increased by about 8%. For W6 12023, this shift in flowering and seed weight corresponded to a threefold increase in winter hardiness. While there is

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**Table 2. Mean seed size as measured by weight (g 100 seed⁻¹), yield per plant (g), height (cm) and branch number at maturity, and percentage flowering across Whitlow and Spillman Farms, Pullman, WA, 2014.** Whole plants were scored for percentage flowering on 16 May, and height, branch number, per plant yield, and 100 seed weight were determined from five representative plants per plot at harvest (16 Aug.). Unselected entries were germplasm stocks of their representative selected W6 accessions. Unselected Wibo/1 seed was sourced from Central Ferry Farm, Central Ferry, WA, 2009–2010.

<table>
<thead>
<tr>
<th>Entry</th>
<th>100 seed weight</th>
<th>Yield per plant</th>
<th>Height at maturity</th>
<th>Branch number</th>
<th>% flowering</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>g</td>
<td>cm</td>
<td>no.</td>
<td>%</td>
</tr>
<tr>
<td>Unselected</td>
<td>WH-1</td>
<td>67.4a</td>
<td>41.3a</td>
<td>64.6a</td>
<td>2.3a</td>
</tr>
<tr>
<td>Selected</td>
<td>58.3a</td>
<td>38.6a</td>
<td>56.9a</td>
<td>1.7a</td>
<td>37.2b</td>
</tr>
<tr>
<td>Unselected</td>
<td>WH-2</td>
<td>59.2a</td>
<td>27.2a</td>
<td>66.9a</td>
<td>1.7a</td>
</tr>
<tr>
<td>Selected</td>
<td>55.5a</td>
<td>26.6a</td>
<td>59.5a</td>
<td>1.6a</td>
<td>20.1bc</td>
</tr>
<tr>
<td>Unselected</td>
<td>WH-3</td>
<td>57.0a</td>
<td>34.6a</td>
<td>66.6a</td>
<td>2.2a</td>
</tr>
<tr>
<td>Selected</td>
<td>55.1a</td>
<td>36.6a</td>
<td>59.3a</td>
<td>1.9a</td>
<td>33.5bc</td>
</tr>
<tr>
<td>Unselected</td>
<td>WH-4</td>
<td>55.6ab</td>
<td>35.6a</td>
<td>59.9a</td>
<td>2.0a</td>
</tr>
<tr>
<td>Selected</td>
<td>57.3ab</td>
<td>32.9a</td>
<td>62.1a</td>
<td>2.1a</td>
<td>26.1bc</td>
</tr>
<tr>
<td>Unselected</td>
<td>Wibo/1</td>
<td>51.4b</td>
<td>22.9a</td>
<td>61.0a</td>
<td>2.1a</td>
</tr>
<tr>
<td>Selected</td>
<td>56.0ab</td>
<td>28.1a</td>
<td>60.5a</td>
<td>2.1a</td>
<td>24.6bc</td>
</tr>
</tbody>
</table>

† Letters separate significant different LS means (P < 0.05).
probably an association between winter hardiness, flowering, and seed size, they appear to be under separate mechanisms of genetic control (Lawes et al., 1983) as indicated by the other selected populations, which only showed shifts in winter hardiness.

As a result of outcrossing (Drayner, 1956) we expected interaccession gene flow over time. Further, the improved performance of these composite populations could be the result of recombination and incorporation of favorable alleles with phenotypic overdominance or simply the enrichment of favorable alleles within the original heterogeneous accessions (Allard, 1960). WH populations have at least maintained seed morphological diversity and, in the case of WH-1, have increased seed morphological diversity based on seed coat color and the decrease in frequency of the recessive clear hilum trait through outcrossing and selection.

We would suggest that researchers interested in this novel source of winter-hardy germplasm should identify an optimal planting window for overwintering faba bean in USDA plant hardiness zone 6b climates and warmer (USDA-ARS, 2012). Alternatively, because these four winter-type faba bean selections are facultative, early spring sowing should also be considered. The productivity of late spring plantings in southeastern Washington is limited by late season drought and heat. However, late maturing winter-type faba bean may hold promise as an early spring sown green manure or cover crop in environments where moisture and temperature are conducive, that is, high temperatures below 30°C during growth and development. Further research should explore the potential of winter-type faba bean as a spring, winter, and even late summer green manure or cover crop.

**Availability**

Small quantities of WH-1, WH-2, WH-3, and WH-4 seed are available for breeders, geneticists, and other research personnel on written request to Jinguo Hu or Clarice Coyne, USDA–ARS, Western Regional Plant Introduction Station, 59 Johnson Hall, Washington State University, Pullman, WA 99164, or through the National Plant Germplasm System (http://www.ars-grin.gov/npgs/orders.html). It is requested that appropriate recognition of the source be made when these germplasm lines are used in the development of novel breeding lines or commercial releases.

**Conclusions**

WH-1, WH-2, WH-3, and WH-4 are the only characterized sources of true winter-hardy faba bean available through the USDA-ARS, Western Regional Plant Introduction Station. These released faba bean lines are expected to complement previously characterized and currently available materials on the seed market. They will be useful for breeding improved pulse and green manure or cover crop faba bean cultivars adapted to the USDA plant hardiness zones of 6b and above.

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Vicia faba

Vicia faba

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