Common bean (Phaseolus vulgaris L.) plays a significant role in diets around the world, especially in developing countries (Messina, 1999). It is high in protein, fiber, vitamins, and minerals and has been linked to preventative effects against diseases such as cancer, diabetes, and heart disease (Anderson et al., 1999). The majority of common bean production occurs in low-input agricultural systems of developing countries (Miklas et al., 2006), and some of the world's production is being relocated to marginal land due to competition for acreage from other crops, such as soybean [Glycine max (L.) Merr.] and maize (Zea mays L.) (Beebe et al., 2013b). With these shifts in production, the crop is being exposed to more environmental stresses. Additionally, enhancing the abiotic stress tolerance of common bean has become more important with the recognition of current and future climate instability. As such, developing cultivars with increased abiotic stress tolerance is the primary goal of bean breeding programs worldwide (Miklas et al., 2006).

Cultivated common bean generally lacks resistance to many abiotic stresses, including drought and subzero temperatures. Tepary bean (P. acutifolius Gray), a relative of common bean, has been reported to be tolerant to these stresses. Preliminary studies screening wild tepary accessions for cold tolerance demonstrated that W6 15578 is a potential donor of cold tolerance, so an interspecific backcross population derived from a cross between it and common bean (NY5-161) was developed. A 3-yr field study was conducted in Saskatoon, SK, to identify lines able to withstand subzero temperatures better than the common bean parent at the seedling stage. Introggression lines were also tested for response to terminal drought under field conditions in Isabela, PR, over 3 yr. Days to flowering and yield measurements, along with subzero temperature and drought stress tolerance data, are presented. Although the interspecific introgression lines were backcrossed twice to common bean to improve the fertility and increase the proportion of common bean genome, several introgression lines performed better than the common bean parent under both stress conditions. Future breeding objectives include backcrossing to tepary to try to recover additional abiotic stress tolerance genes, as well as using selected introgression lines as breeding material to develop common bean varieties with increased subzero temperature stress and drought stress tolerance. Interspecific introgression of portions of the tepary bean genome into common bean is a promising method for increasing abiotic stress tolerance in common bean.

Successful Introgression of Abiotic Stress Tolerance from Wild Tepary Bean to Common Bean

Jodi R. Souter, Valarmathi Gurusamy, Timothy G. Porch, and Kirstin E. Bett*

ABSTRACT
Common bean (Phaseolus vulgaris L.) production is limited due to abiotic stresses, including drought and subzero temperatures. Tepary bean (P. acutifolius Gray), a relative of common bean, has been reported to be tolerant to these stresses. Preliminary studies screening wild tepary accessions for cold tolerance demonstrated that W6 15578 is a potential donor of cold tolerance, so an interspecific backcross population derived from a cross between it and common bean (NY5-161) was developed. A 3-yr field study was conducted in Saskatoon, SK, to identify lines able to withstand subzero temperatures better than the common bean parent at the seedling stage. Introggression lines were also tested for response to terminal drought under field conditions in Isabela, PR, over 3 yr. Days to flowering and yield measurements, along with subzero temperature and drought stress tolerance data, are presented. Although the interspecific introgression lines were backcrossed twice to common bean to improve the fertility and increase the proportion of common bean genome, several introgression lines performed better than the common bean parent under both stress conditions. Future breeding objectives include backcrossing to tepary to try to recover additional abiotic stress tolerance genes, as well as using selected introgression lines as breeding material to develop common bean varieties with increased subzero temperature stress and drought stress tolerance. Interspecific introgression of portions of the tepary bean genome into common bean is a promising method for increasing abiotic stress tolerance in common bean.

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Abbreviations: HI, harvest index; LD50, temperature estimated to cause 50% mortality; MS, Murashige and Skoog; RCBD, randomized complete block design.

COMMON bean (Phaseolus vulgaris L.) plays a significant role in diets around the world, especially in developing countries (Messina, 1999). It is high in protein, fiber, vitamins, and minerals and has been linked to preventative effects against diseases such as cancer, diabetes, and heart disease (Anderson et al., 1999). The majority of common bean production occurs in low-input agricultural systems of developing countries (Miklas et al., 2006), and some of the world’s production is being relocated to marginal land due to competition for acreage from other crops, such as soybean [Glycine max (L.) Merr.] and maize (Zea mays L.) (Beebe et al., 2013b). With these shifts in production, the crop is being exposed to more environmental stresses. Additionally, enhancing the abiotic stress tolerance of common bean has become more important with the recognition of current and future climate instability. As such, developing cultivars with increased abiotic stress tolerance is the primary goal of bean breeding programs worldwide (Miklas et al., 2006).

Cultivated common bean generally lacks resistance to many abiotic stresses, including water limitation (or drought stress), which affects >60% of dry bean production worldwide (Rao et al., 2013). Bean production is greatly affected by both terminal and intermittent drought; therefore, much of the breeding to


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increase tolerance has focused on traits such as earliness and partitioning toward reproductive structures that are correlated with yield under drought (Beaver et al., 2003).

In northern regions of bean production, such as the northern Great Plains, cold temperatures force late planting and early harvest, thus shortening the growing season and impeding yield. Much of the cold stress tolerance research reported in bean has focused on germination at low temperatures. Common bean has limited germination below 15°C (Dickson and Boettger, 1984), which greatly limits the number of frost-free days in the production cycle in many areas. Subzero temperature stress tolerance after germination is also important, as early-seeded, cold-germinating lines risk a late frost event. Subzero temperature stress at the seedling stage is very damaging to common bean. In a study with 10 species of legumes, the two common beans were the least tolerant to subzero temperatures at the seedling stage (Meyer and Badaruddin, 2001). Meyer and Badaruddin (2001) also found that the unifoliate and the first trifoliolate leaf stages were the most sensitive to freezing temperatures in common bean. Their research concluded that bean had a temperature estimated to cause 50% mortality (LD50) of −3.25°C, although regrowth after survival was limited, meaning few plants make it to maturity. Another study by Ashworth et al. (1985) suggested a LD50 of −2.7°C, with the first ice nucleation event occurring at −1.3°C. An increase in tolerance of only 2 to 3°C would allow for expansion of dry bean production into areas with shorter growing seasons, including the Canadian prairies and higher altitudes in the tropics (Balasubramanian et al., 2004b).

Modern common bean germplasm has a restricted genetic base (Papa and Gepts, 2003), and other tactics have to be employed to increase genetic variability with respect to tolerance to constraints like abiotic stress (Nabhan and Felger, 1978; Kelly, 2010). Untapped crop relatives, like tepary bean and other closely related species, need to be explored to understand novel genetic variation, which may assist in increasing abiotic stress tolerance in common bean (Porch et al., 2013).

Tepary bean (Phaseolus acutifolius Gray), a relative of common bean, was first identified in Northwestern Mexico and Southern Arizona (Nabhan and Felger, 1978; Kelly, 2010). It is a subsistence crop in areas of the southern United States and northern Mexico; however, there have been few tepary bean improvement efforts in largescale breeding programs due to the currently low consumption worldwide (Bhardwaj et al., 2002). Tepary bean has a similar nutritional composition to common bean (Porch et al., 2016). Due to its high protein content, high productivity, adaptation to arid environments, and a wide profile of disease resistance, research on tepary bean is warranted, including its use as a donor of new genetic variability to Phaseolus species (Nabhan and Felger, 1978). Interspecific crosses between common bean and tepary bean have already been successfully used to transfer common bacterial blight [caused by Xanthomonas axonopodis pv. phaseoli (Smith) Vauterin et al.] resistance genes (Thomas and Waines, 1984; Parker and Michaels, 1986; Singh and Muñoz, 1999) and heat resistance genes (CIAT, 2015). Because only a small portion of genetic variability in tepary bean has been used for common bean improvement, there is still potential for large gains to be made through interspecific gene transfer (Singh, 2001).

In particular, interspecific breeding to transfer genes associated with abiotic stress tolerance from tepary to common bean needs exploring. Tepary bean is more resistant to high temperature stress (Gaur et al., 2015), subzero temperature stress (Balasubramanian et al., 2004b; Martinez et al., 2007), salt stress (Goertz and Coons, 1991), and drought stress (Beebe et al., 2013a) than common bean. Tepary bean can withstand drought conditions much better than common bean (Thomas et al., 1983), although differences in the response to drought do occur among tepary bean accessions (Mohamed et al., 2002).

Tepary bean tolerance to low temperatures is not apparent at germination, with tepary bean taking longer to germinate at lower temperatures compared with common bean (Scully and Waines, 1987); however, increased tolerance is apparent at the seedling stage. Balasubramanian et al. (2004b) observed a tepary accession (PI 535248) with leaflets having an LD50 0.5°C lower than that of any of the common beans studied in the field and at least a 1.0°C lower in a controlled environment (Balasubramanian et al., 2004b). Further experiments by Martinez et al. (2007) showed two other wild tepary accessions (PI 219445 and W6 15578) that had greater tolerance to subzero temperatures than common beans studied, including NY5-161, a common bean cultivar previously categorized as tolerant to cold temperatures (Dickson and Boettger, 1984).

Tepary bean falls into the tertiary genepool of common bean, meaning crosses between the two require embryo rescue and one or more backcrosses are needed to restore fertility (Singh, 2001). While there can be issues with incompatibility (Parker and Michaels, 1986) and negative nuclear-cytoplasmic interactions (Waines et al., 1988), judicious selection of the parental genotypes can usually resolve these issues.

Here, we report on the development of an interspecific backcross population using a stress-tolerant wild tepary bean accession (W6 15578) and a receptive common bean breeding line (NY5-161) and the evaluation of the introgression lines for response to drought and subzero temperatures and for agronomic traits.

MATERIALS AND METHODS

Plant Material

Common bean genotype NY5-161 is a determinate bush-type breeding line of Andean background developed by M. Dickson

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at Cornell University (Geneva, NY) that can mature in Saskatoon, SK. NY5-161 was reported to be more tolerant to cold stress than other beans (Holubowicz and Dickson, 1989). A wild tepary bean *P. acutifolius* var. *acutifolius* accession W6 15578, originating in Mexico and accessed from the USDA-ARS Plant Germplasm Introduction and Testing Research Unit in Pullman, WA, has been identified as more tolerant to subzero temperatures than several common bean lines (Martinez et al., 2007; Martinez, 2010). It has an indeterminate growth habit and is photoperiod sensitive and thus does not flower until the end of the growing season in Saskatoon. The seed size of W6 15578 is much smaller than that of NY5-161 and, as a wild accession, it must be scarified to induce water uptake and germination. Interspecific crosses were made between these two lines, with NY5-161 as the female parent in all crosses. One hundred and eight embryos were rescued at the late heart stage on a medium containing 0.75 strength Murashige and Skoog and Skoog (MS) media, B5 vitamins (consisting of 100 μg L\(^{-1}\) myoinositol, 1 mg L\(^{-1}\) nicotinic acid, 0.1 mg L\(^{-1}\) pyridoxine HCl, and 10 mg L\(^{-1}\) thiamin HCl), abscisic acid (ABA, 0.5 mg L\(^{-1}\)), 3% sucrose, and 0.5% agar. Embryos with a developing shoot axis were transferred to a shoot culture media containing MS media (full strength), B5 vitamins, 6-benzyladenine (BA, 0.5 mg L\(^{-1}\)), 3% sucrose, and 0.6% agar. Three F\(_1\) plants resulted that were male sterile and had reduced female fertility, and one was backcrossed with pollen from NY5-161. The embryos from the BC\(_1\) were rescued using the abovementioned protocol, which yielded three BC\(_1\) offspring. Each were again backcrossed with pollen parent, NY5-161, resulting in six BC\(_2\)\(_F_1\) individuals (named A–F). No rescuing was required at this stage. Sufficient seed to produce up to 15 individuals from each of the BC\(_2\)\(_F_1\) plants were planted to produce BC\(_2\)\(_F_2\) populations. Seed was harvested from all plants that matured, and 10 seeds of each BC\(_2\)\(_F_2\) were sown indoors. Cuttings were made for seed increase, and the mother plants were transplanted to the field in Saskatoon on 12 May 2009 and covered to protect from low temperatures for a week. Seed from surviving plants were bulked at the end of the season to produce populations that could be used for more thorough agronomic assessments. The lines were advanced one more generation, and selection for plant growth habit and maturity was emphasized.

### Agronomic Trials

Early-maturing common bean cultivars CDC Pintium and CDC Expresso, along with the parents of the introgression lines, were used as checks in all agronomic trials. Four tepary bean cultivars obtained from Prairie Garden Seeds, Humboldt, SK, were also included as checks. All the trials were conducted in Saskatoon (52°07’22.0”N 106°37’22.4” W in 2013 and 52°08’12.4” N 106°37’13.8” W in 2014 and 2015; ~481 m asl; Dark Brown soil zone). A total of 136 lines, including 17 accessions of tepary bean, 3 common beans, and 116 adapted introgression lines, were evaluated in a RCBD with four replications. Each line was planted in a single 1-m row with a four-row seeder (30-cm spacing) on three separate planting dates in mid- to late August, in an attempt to subject the population to a subzero temperature event at the seedling stage. This would mimic the physiological stage that would incur a subzero temperature event in a late spring frost, but without the confounding problem of germination in cold soil. After each subzero temperature event, seedlings at approximately the V1 stage (first trifoliate unfolded) were assessed. Counts of number of plants germinated and number of plants alive or dead were recorded 1 and 7 d after the frost event. In 2013, plants were rescued from the field after the frost and were grown in a greenhouse to confirm survival and to increase seed. Ambient air temperatures were recorded using a Hobo Pro V2 data logger (Onset).

### Subzero Temperature Trial

Subzero temperature trials were conducted in three consecutive years (2013–2015) in fields in the vicinity of Saskatoon (52°07’22.0”N 106°37’22.4” W in 2013 and 52°08’12.4” N 106°37’13.8” W in 2014 and 2015; ~481 m asl; Dark Brown soil zone). A total of 136 lines, including 17 accessions of tepary bean, 3 common beans, and 116 adapted introgression lines, were evaluated in a RCBD with four replications. Each line was planted in a single 1-m row with a four-row seeder (30-cm spacing) on three separate planting dates in mid- to late August, in an attempt to subject the population to a subzero temperature event at the seedling stage. This would mimic the physiological stage that would incur a subzero temperature event in a late spring frost, but without the confounding problem of germination in cold soil. After each subzero temperature event, seedlings at approximately the V1 stage (first trifoliate unfolded) were assessed. Counts of number of plants germinated and number of plants alive or dead were recorded 1 and 7 d after the frost event. In 2013, plants were rescued from the field after the frost and were grown in a greenhouse to confirm survival and to increase seed. Ambient air temperatures were recorded using a Hobo Pro V2 data logger (Onset).

### Water Limitation Trial

Water limitation trials were conducted in the winters of 2013, 2014, and 2015 at the USDA-ARS field site in Isabela, PR (18°28’18.3”N and 67°3’19.1” W; 122 m asl). The trials were planted in a split plot design with water treatment as the main plot and genotype as the subplot. The trials consisted of four common beans, three tepary beans, and the introgression lines. The number of introgression lines depended on seed availability: 117 in 2013, 89 in 2014, and 86 in 2015. There were two, three, and four replications in 2013, 2014, and 2015, respectively. Approximately 45 seeds were planted in 2.74-m rows, spaced 0.76 m apart, with a border row surrounding the trial. Planting occurred in late January, and the trial was irrigated as needed before the treatment period. Irrigation was discontinued in the water limitation plots at the initiation of flowering (before 4 wk after planting), whereas the control plots were irrigated twice a week thereafter. Flowering dates, germination counts, yield, HI (2013), and days to flowering (2014 and 2015) were recorded. In 2014, yield in a section of the irrigated block was reduced due to poor germination. Therefore, yield was first corrected on the basis of percentage germination. To avoid edge effects, only the inner 2 m of each plot was harvested for yield and biomass measurements. Intensity of the drought was measured by the drought intensity index (DII = 1 − average yield\(_{\text{stress}}\)/average yield\(_{\text{nonstress}}\)), where

\[ \text{DII} = 1 - \frac{\text{average yield}_{\text{stress}}}{\text{average yield}_{\text{nonstress}}} \]

Days to flowering was noted when at least 50% of the plants had at least one flower opened, and days to maturity was noted when all the pods had attained physiological maturity. To measure harvest index (HI), plants were uprooted and dried, then weighed and threshed to determine seed yield. The HI was estimated as (seed dry weight/whole plant dry weight) × 100.

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Yi,N and Yi,S are the yields of not stressed and stressed individuals, respectively (Rosielle and Hamblin, 1981).

Climatic data were collected from the USDA-NRCS website (www.wcc.nrcs.usda.gov) from data measured at the Isabela substation. If data were incomplete, measurements from the Mora Camp, PR, substation and the Coloso, PR, substation were used.

Statistical Analysis
Genotypes were analyzed separately, as well as combined into classes—tepary, common, or introgression—to compare across type. The SAS system 9.4 (SAS Institute, 2013) was used for all statistical analyses. Unless otherwise indicated, data are presented as averages ± standard error and comparison at the 95% confidence interval using a post hoc Tukey test. One- and two-way ANOVA were used to assess the effects of factors and the interaction of factors (where appropriate). PROC GLM was used for all ANOVAs. Levene’s test revealed heterogeneous variance of the trial data; therefore, each trial was analyzed separately. Figures were developed using R (R Core Team, 2016).

RESULTS
Agronomic Traits
Agronomic assessments in 2011 revealed variation among the introgression lines for days to flowering and maturity (Fig. 1). Both measurements had significant genotype (p < 0.001) and class (p < 0.001) effects, but among-class differences were only significant for maturity (p = 0.04).

In 2015, tepary beans took significantly longer to flower than both the common beans and introgression lines (p < 0.05, Fig. 2a), and the common beans matured significantly earlier than both the tepary beans and introgression lines (p < 0.05, Fig. 2b). Harvest index also differed among genotypes (p < 0.001) and class, with the common bean HI being statistically greater than that of the introgression lines (p < 0.05, Fig. 2c and Table 1). No statistical differences existed for yield (p = 0.08, Fig. 2d). For all measurements, the introgression class had extensive variation, including several outliers, which suggested that further exploration of some of the introgression lines would be promising.

Tolerance to Subzero Temperature Exposure
The first significant subzero temperature events over years ranged from 12 September to 3 October, and all data were from 24- to 34-d-old plants (Supplemental Table S1). Minimum temperatures and duration of subzero temperature exposure (Supplemental Table S1) varied between plantings and likely account for the wide variability in results from test to test. The lowest initial subzero temperature event occurred in 2013, when the first damaging frost was −4.6°C. This event was also the longest in duration, with subzero temperatures lasting for 12.5 h. The least severe event affected the first planting in 2014, when temperatures...

Fig. 1. Days to flowering and maturity ranking in Saskatchewan, 2011. (a) Days to flowering and (b) maturity ranking (scored 1–5, with 5 being the most mature) of the 348 introgression lines and three common beans in a trial in Saskatoon, SK, in 2011. The arrow beside the common bean column indicates the average of the common bean parent, NY5-161.
Survival differed among genotypes ($p < 0.001$) and classes ($p < 0.001$) (Fig. 3d). Tepary beans had a higher percentage survival (60.6%) than common beans (7.1%) and introgression lines (10.2%) ($p < 0.05$, Table 2).

The subzero temperature stress in 2013 killed almost the entire set of germplasm (Fig. 3a), and there was no difference in survival among genotypes. Although survival was not significant among genotypes, there was a significant difference among classes ($p < 0.001$), with tepary beans demonstrating a higher percentage survival than the introgression lines (Table 2). In the first subzero trial in 2014, >99% of plants were still alive 1 d after frost. Therefore, data are only presented for survival 7 d after the frost event. Survival differed among genotypes ($p < 0.001$) and classes ($p < 0.001$) (Fig. 3d). Tepary beans had a higher percentage survival (60.6%) than common beans (7.1%) and introgression lines (10.2%) ($p < 0.05$, Table 2).

Table 1. Harvest index of common and tepary beans and introgression lines grown in Saskatchewan and Puerto Rico. The Saskatchewan 2015 trial included 116 introgression lines, 3 common beans, and 4 tepary beans, whereas the Puerto Rico 2013 trial included 117 introgression lines, 3 common beans, and 4 tepary beans. Values presented are means.

<table>
<thead>
<tr>
<th>Class</th>
<th>Saskatchewan 2015</th>
<th>Drought-stressed</th>
<th>Well-watered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common bean</td>
<td>52.1a†</td>
<td>41.0a</td>
<td>41.7a</td>
</tr>
<tr>
<td>Introgression lines</td>
<td>42.5b</td>
<td>30.1b</td>
<td>30.1b</td>
</tr>
<tr>
<td>Tepary bean</td>
<td>41.7ab</td>
<td>43.0a</td>
<td>25.2b</td>
</tr>
</tbody>
</table>

† Lowercase letters indicate differences in the columns at $p < 0.05$. Only reached $-1.5^\circ C$. The shortest subzero temperature event occurred in 2015, when temperatures were only below freezing for 2.5 h (Supplemental Table S1). Two tests were analyzed in 2014, as the second test had not fully emerged at the time of the first subzero temperature event. Analysis of variance was completed on the 137 genotypes, and group analysis was completed on the three classes: common bean, tepary bean, and introgression lines.

Fig. 2. Agronomic measurements in the 2015 Saskatoon trial: (a) days to flowering, (b) days to maturity, (c) harvest index, and (d) plot yield of 116 introgression lines, 3 common beans, and 4 tepary beans in a trial in Saskatoon, SK, in 2015. The arrow beside the common bean column indicates the average of the common bean parent, NY5-161.
Survival 1 and 7 d after subzero temperature exposure was significantly different among genotypes in the second trial of 2014 ($p < 0.001$, Fig. 3e and 3f). One day after subzero exposure, the tepary beans had the highest percentage survival compared with the introgression lines and common beans ($p < 0.05$, Table 2). After 7 d, the tepary beans still had the greatest percentage survival at 41.6% ($p < 0.05$, Table 2). At neither time point was survival of common beans and introgression lines statistically different. One day after the 2015 subzero temperature stress event, survival rates between genotypes were not different, but they were significantly different 7 d after exposure ($p < 0.001$).

Differences were significant among classes both 1 and 7 d after the stress event (Fig. 3g and 3h, Table 2). Tepary beans had the highest average survival (35.3%), significantly higher than the introgression lines, but not the common beans ($p < 0.05$, Table 2). Survival of common beans and introgression lines were not statistically different in 2015. In all trials, however, there were individual introgression lines that were just as tolerant as the tepary beans 7 d after exposure. Introgression lines that showed promising rates of survival included A-3-5, D-13-4Br, E-6-2, B-1-4, and D-6-13 (Supplemental Table S2).

**Fig. 3.** Percentage survival of genotypes 7 d after the subzero temperature event. Survival of genotypes 1 (a, c, e, and g) and 7 d (b, d, f, and h) after subzero temperature stress in 2013 (a and b), in the first trial in 2014 (c and d), in the second trial in 2014 (e and f), and in 2015 (g and h). Plantings included four replicates of 17 tepary beans, 3 common beans, and 116 introgression lines. Surviving genotypes in 2013 were rescued from the field 2 d after the frost, so no 7-d data were recorded. One day after the frost in 2014, >99% of genotypes survived, so no data were reported. The arrow beside the common bean column indicates the average of the common bean parent, NY5-161.

**Table 2.** Survival in subzero temperature trial in Saskatoon, SK. Data are means of four replicates of 17 tepary beans, 3 common beans, and 116 introgression lines.

<table>
<thead>
<tr>
<th>Days after frost event</th>
<th>Class</th>
<th>2013</th>
<th>2014 First Planting</th>
<th>2014 Second Planting</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Common bean</td>
<td>0.6 ± 0.6ab†</td>
<td>N/A‡</td>
<td>36.1 ± 5.4a</td>
<td>87.3 ± 6.3ab</td>
</tr>
<tr>
<td></td>
<td>Introgression lines</td>
<td>1.0 ± 0.2a</td>
<td>N/A</td>
<td>36.9 ± 0.8a</td>
<td>86.9 ± 0.8a</td>
</tr>
<tr>
<td></td>
<td>Tepary bean</td>
<td>3.7 ± 0.8b</td>
<td>N/A</td>
<td>77.2 ± 2.7b</td>
<td>95.6 ± 1.5b</td>
</tr>
<tr>
<td>7</td>
<td>Common bean</td>
<td>N/A</td>
<td>7.1 ± 2.4a</td>
<td>25.0 ± 4.5a</td>
<td>20.8 ± 3.0a§</td>
</tr>
<tr>
<td></td>
<td>Introgression lines</td>
<td>N/A</td>
<td>10.2 ± 0.6a</td>
<td>22.5 ± 0.7a</td>
<td>28.3 ± 0.9a§</td>
</tr>
<tr>
<td></td>
<td>Tepary bean</td>
<td>N/A</td>
<td>60.6 ± 6.4b</td>
<td>41.6 ± 4.0b</td>
<td>35.3 ± 2.5b§</td>
</tr>
</tbody>
</table>

† Numbers in the same column with different letters are significantly different at $p < 0.05$.
‡ N/A = data not available for this time point.
§ Welch’s ANOVA was used due to dissimilar standard deviations.
Tolerance to Water Limitation

Calculations of drought intensity index (1 − average yield-
\(\frac{\text{Stressed}}{\text{average yield}_{\text{Nonstressed}}} \)) demonstrated that the stress was moderate in 2013 and mild in 2014 and 2015 (Supplemental Table S3). Although differences existed in the level of stress from year to year, each year, the effect of stress was significant (\(p < 0.001 \)). Classifying genotypes into tepary bean, common bean, or introgression lines revealed significant yield differences among these classes (\(p < 0.001 \)). In 2013 and 2014, there was a significant interaction between the stress and the genotypes (\(p < 0.001 \)) and classes (\(p < 0.01 \) and \(p < 0.001 \), respectively). In all 3 yr, the introgression lines showed a range of responses, with several having greater yield than NY5-161 under drought stress.

Although most genotypes and classes had reduced yield under stress, the tepary beans and several introgression lines increased yield relative to the well-watered control, especially in 2013 (Table 3). Individually in 2013, the average yield of the four tepary bean cultivars (Tepary Gray, Tepary White, Sonoran Gold, and G40001) and three of the introgression lines (D-5-10, D-1-5, and C-11-2Pattern) increased under water limitation. Most introgression lines yielded lower than the common beans, including the common bean parent NY5-161, under both irrigated and stress conditions, but a few had comparable yields (Fig. 4).

Two indices (geometric mean and stress tolerance) were used to rank the water limitation tolerance of the population, and the tepary beans generally performed better than the common beans and the introgression lines, regardless of the index used (Supplemental Table S4). The rankings of NY5-161 and some of the introgression lines varied, depending on whether the index used measured tolerance or yield stability (Supplemental Table S4).

In the water limitation trial, differences in days to flowering existed among genotypes (Fig. 5b and 5c). The introgression lines segregated according to the BC\(\text{2F}_1\) from which they originated, with the B-, C-, and F-derived lines behaving similarly to NY5-161, whereas the A- and D-derived lines flowered later than the common bean parent (\(p < 0.05 \), Table 4).

Harvest index was measured in 2013, and there were differences among genotypes and classes (\(p < 0.001 \)). The introgression lines had a lower HI than the common beans in both well-watered and drought-stressed conditions and a lower HI than the tepary beans in the drought stressed condition (\(p < 0.05 \), Table 1). The tepary beans had a higher HI under drought stress conditions than under well-watered conditions (\(p < 0.01 \), Fig. 5a).

### DISCUSSION

Stand establishment failure due to susceptibility to early seedling freezing temperatures is deleterious to legume production (Badaruddin and Meyer, 2001). This is especially the case in common bean, as the growing point is above the ground and the plant will not regrow after damage. In our study, <30% of the common bean checks survived even a mild subzero temperature event (Fig. 3); therefore, any increase in tolerance to subzero temperatures at the seedling stage would be beneficial. Balasubramanian et al. (2004b) studied wild relatives of common bean and survival after subzero temperature stress. Although the one tepary bean accession studied did not survive at the lowest temperatures survived by several wild relatives of common bean, including P. \textit{filiformis} Benth., \textit{P. angustissimus} A. Gray, and \textit{P. ritenis} M.E. Jones, it still survived temperatures lower than those of the three common beans studied, including CDC Blackhawk, which was previously shown to have tolerance to suboptimal temperatures during emergence (Balasubramanian et al., 2004a, 2004b). Later, two other tepary accession were documented to have greater tolerance than the common beans studied (Martinez et al., 2007), and one of these was used to develop the introgression lines studied here. We confirmed that tepary beans have a higher tolerance to subzero temperature stress than common beans under field conditions (Table 2, Fig. 3). The response of the genotypes studied varied, but in three frost events, the survival of tepary beans as a group had a higher mean percentage survival than the common beans. The lethal temperatures documented by Balasubramanian et al. (2004b) were lower than the temperatures that the seedlings were exposed to in this study, but since that study was done in chambers and ours was done in the field, the environmental component, including light and wind, would have affected ice nucleation and overall plant health. Also,

<table>
<thead>
<tr>
<th>Class</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Limited</td>
<td>Control</td>
<td>Limited</td>
</tr>
<tr>
<td>Common bean</td>
<td>827.0 ± 54.8Ax†</td>
<td>1244 ± 73.5Bx</td>
<td>1258.5 ± 137.8Ax</td>
</tr>
<tr>
<td>Introgression lines</td>
<td>493.16 ± 14.4Ay</td>
<td>857.3 ± 20.7By</td>
<td>676.2 ± 15.9Ay</td>
</tr>
<tr>
<td>Tepary bean</td>
<td>1039.3 ± 100.6Ax</td>
<td>846.75 ± 100.2Bxy</td>
<td>1904.9 ± 259.5Az</td>
</tr>
<tr>
<td>Total populations</td>
<td>523.0 ± 15.7A</td>
<td>869.9 ± 19.9B</td>
<td>748.9 ± 24.1A</td>
</tr>
</tbody>
</table>

† Uppercase letters signify differences between water-limited and control in a given year, and lowercase letters indicate differences among the groups within a year and treatment (\(p < 0.05 \)).
Fig. 4. Yield under conditions of water limitation stress. Yield (kg ha⁻¹) of the introgression lines, common beans, and tepary beans after a water limitation trial in Isabela, PR, in (a) 2013, (b) 2014, (c) and 2015. WW, well-watered (control); DS, drought-stressed. Means of 117, 89, and 86 introgression lines were included in 2013, 2014, and 2015, respectively, with four common beans and three tepary beans included in all years. The arrow beside the common bean column indicates the average of the common bean parent, NY5-161.

Age effects and organ studied (leaf versus whole plant) may have contributed to the difference. The stage of our plants, the unifoliate leaf stage and the first trifoliolate leaf stage, were found to be the most sensitive to subzero temperature stress in common bean (Meyer and Badaruddin, 2001). Therefore, leaf age may have also contributed to differences in survival temperatures between our study and that of Balasubramanian et al. (2004b).

Survival rates varied among the four trials analyzed (Fig. 3, Table 2), which was not surprising, as it has been documented that tolerance measurements vary due to the intensity and duration of the stress, the rate of stress development, and the phenological timing of the stress (Srinivasan et al., 1998). The life stage was similar from year to year; however, the intensity, duration, and rate of the stress varied considerably (Supplemental Table S1). The importance of following up on survival several days after exposure to subzero temperatures was clear in this analysis. It has been noted that, in plants with little to no freezing tolerance on the morning after the frost, the injured plant looks flaccid and water soaked, as cell membranes have lost their semipermeability and the intracellular compartmentalization is destroyed (Burke et al., 1976). The plants are not dead but will eventually die due to these symptoms. Regrowth after subzero stress events differs, depending on the temperatures reached and the age of the bean plants (Meyer and Badaruddin, 2001). In our trials, seedlings were often still green 1 d after stress but succumbed to their injuries within 7 d after the stress (Fig. 3). This phenomenon has been previously noted.
Fig. 5. Harvest index and days to flowering of the introgression lines. Harvest index from (a) 2013 and days to flowering of the introgression lines, four common beans, and three tepary beans after a water limitation trial in Isabela, PR, in (b) 2014 and (c) 2015. Means of 117, 89, and 86 introgression lines were included in 2013, 2014, and 2015, respectively. WW, well-watered (control); DS, drought-stressed. The arrow beside the common bean column indicates the average of the common bean parent, NY5-161.

Table 4. Mean days to flower (DTF) of progeny derived from single $BC_2F_1$ individuals in Saskatchewan and Puerto Rico.

<table>
<thead>
<tr>
<th>$BC_2F_1$ population†</th>
<th>Saskatchewan</th>
<th>Puerto Rico</th>
<th>Saskatchewan</th>
<th>Puerto Rico</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>55.3</td>
<td>41.1</td>
<td>32.7‡</td>
<td>30.6‡</td>
</tr>
<tr>
<td>B</td>
<td>53.5</td>
<td>40.6</td>
<td>31.4</td>
<td>28.5</td>
</tr>
<tr>
<td>C</td>
<td>55.7</td>
<td>41.3</td>
<td>32.3</td>
<td>28.6</td>
</tr>
<tr>
<td>D</td>
<td>54.6</td>
<td>41.6</td>
<td>32.6‡</td>
<td>30.8‡</td>
</tr>
<tr>
<td>E</td>
<td>53.8</td>
<td>40.5</td>
<td>32.7‡</td>
<td>30.6‡</td>
</tr>
<tr>
<td>F</td>
<td>51.8</td>
<td>38.9</td>
<td>31.7</td>
<td>28.6</td>
</tr>
<tr>
<td>Common bean</td>
<td>52.0</td>
<td>40.7</td>
<td>31.2</td>
<td>27.5</td>
</tr>
<tr>
<td>Tepary bean</td>
<td>–</td>
<td>53.6‡</td>
<td>31.8</td>
<td>28.7</td>
</tr>
</tbody>
</table>

† Saskatchewan 2011 included 60 As, 82 Bs, 30 Cs, 119 Ds, 46 Es, 14 Fs, and 3 common beans. Saskatchewan 2015 included 25 As, 32 Bs, 6 Cs, 29 Ds, 19 Es, 5 Fs, 4 tepary beans, and 3 common beans. Puerto Rico data included 23 As, 26 Bs (25 in 2015), 3 Cs, 19 Ds, 13 Es, 5 Fs, 4 tepary beans, and 3 common beans.

‡ Statistically different in mean DTF from the common bean parent, NY5-161 ($p < 0.05$).
with tepary beans under drought conditions, and it has been hypothesized that tepary beans rely on stress postponement more so than tolerance (Markhart, 1985).

Two tepary bean accessions with tolerance to water limitation superior to that of the common beans studied had previously been identified in an experiment that compared yield under irrigation and yield under rain-fed (water-limited) conditions (Rao et al., 2013). According to the data from our study, tepary beans exhibited greater yield than common bean after a terminal water limitation stress had been imposed (Fig. 4). Tepary beans often did better under the mild to moderate stress conditions than the irrigated conditions, suggesting either that excessive irrigation hampers tepary bean performance or that stress signals encourage indeterminacy of tepary bean, resulting in increased reproduction. To the best of our knowledge, no data have been published analyzing the response of tepary bean to water logging, although high water availability is known to cause excessive vegetative growth in indeterminate plants (De Costa et al., 1997), a growth habit common to the tepary beans in this trial. Bean plants with indeterminate growth habits have an inherent flexibility in quantity of flowers formed (Nleya et al., 1999), which allows the plant to take advantage of opportune times for growth and reproduction while delaying these processes during inopportune conditions. Rosales-Serna et al. (2004) found that indeterminate bean cultivars had greater seed yield under drought than the determinate cultivars studied. Indeterminate bean cultivars demonstrated greater yield stability than determinate bean cultivars in an experiment of 42 trials differing in climate and soil conditions over a 5-yr period in Michigan (Kelly et al., 1987). Similar terminal drought stress observations have been documented in other plants such as faba bean (Vicia faba L.), as it has been determined that the greater flexibility offered by an indeterminate growth habit can exploit midseason water inputs (De Costa et al., 1997). Although on average indeterminate introgression lines were limited in the water limitation trial, several showed promise with high stress tolerance rankings in 2013, including C-11-2Pattern and C-6-5. Unfortunately, these lines were not included in later trials.

The hypothesis that introgression of abiotic stress tolerance traits from tepary bean into common bean is possible was supported by the identification of several introgression lines having superior tolerance to subzero temperature stress (Fig. 3) and water limitation stress (Fig. 4) over the common bean parent. For example, lines B-7-7Br and D-13-4Blk had high levels of drought tolerance in all years; D-7-2Br, E-6-3, A-11-7, and B-5-11 showed high yield stability (geometric mean) over all years (Supplemental Table S4); and A-3-5 had superior survival after subzero temperature stress in all years. Lines D-13-4Br, E-6-2, B-1-4, and D-6-13 also show promise as sources of tolerance to subzero temperature stress, with high rankings in at least 2 of the 3 yr (Supplemental Table S2). No introgression line was superior to the other introgression lines under both water limitation and subzero temperature stress; however, several lines that were superior during one stress were also better than NY5-161 during the other stress, including D-14-4Blk, E-6-3, D-13-4Br, and B-1-4. The agronomic performance and stress response of the interspecific introgression lines varied greatly among the introgression lines (Fig. 3, 4, and 5). Some introgression lines performed well, but there was a lot of transgressive segregation observed in this population. This is consistent with populations derived from other interspecific hybridizations, where extreme traits have been observed to be the norm and with 91% of studies reporting at least one transgressive trait (Rieseberg et al., 1999). Unfortunately, in this population, the transgressive segregation observed was mainly in the form of undesirable traits, (e.g., longer days to flowering and maturity; Table 4, Fig. 2).

The difficulties of crossing common bean and tepary bean include early-stage embryo rescue of embryos 7 to 10 d old, embryo rescue in two stages, and male sterile offspring with some level of female sterility. As such, the introgression lines used in these experiments had been backcrossed to the common bean parent twice to recover full female and male fertility; therefore, they have predominately common bean genomes. Out of necessity, they were also selected to mature in Saskatoon, which likely further skewed the genome towards the more adapted common bean parent. For instance, any BC$_2$F$_2$ individual that had secondary dormancy or was photoperiod sensitive (traits from the wild tepary parent) did not reproduce. Therefore, it was not surprising that, by the BC$_2$F$_4$, a large portion of the introgression population was phenotypically very similar to the common bean parent.

With phenotypic information gained during this study on the available cultivated tepary bean population, such as yield potential and abiotic stress tolerance, future interspecific introgression populations could be developed through congruity backcrossing with a more suitable tepary bean parent and, therefore, have a greater chance of success of increasing abiotic stress tolerance in common bean. Notably, the lines Tepary Gray and Sonoran Gold, which are adapted to Saskatchewan growing conditions, were higher yielding under both control and water-limited conditions and had superior survival after subzero temperature stress than the common bean checks, the interspecific introgression lines, and most of the other tepary beans studied. These locally adapted lines could be used in further crosses to the superior introgression lines for further use of the tepary genome to improve abiotic stress tolerance in common bean.
Conflict of Interest
Authors have no known conflicts of interest in the submission of this article.

Supplemental Material Available
Supplemental material for this article is available online.

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