Evaluation in Vitro of Blueberry Germplasm for Higher pH Tolerance

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Abstract. Progenies from crosses among eight highbush (*Vaccinium corymbosum* L.), lowbush (*V. angustifolium* Ait.), and *V. corymbosum*/*V. angustifolium* hybrid-derivative parents were evaluated in vitro at low (5.0) and high (6.0) pH for vitality, height, and dry weight. Succinic acid and 2[N- morpholino]ethanesulfonic acid (Mes) effectively maintained pH in the medium and rhizosphere. The pH regime did not affect percent radicle emergence from seed or survival; however, percent seed germination was slightly lower at high pH. The parental general combining ability (GCA), reciprocal and maternal, but not the specific combining ability (SCA) variance components were significant for plant vitality, height, and dry weight. The GCA variance components were six to 26 times larger than the SCA variance components for the plant growth traits. Variation due to pH regime was significant for vitality and dry weight but not for plant height. The progenies of parents with high percent lowbush ancestry were taller at both pH levels than those with less such ancestry. Little variation was apparent for higher pH tolerance as measured by dry weight; however, the GCA effects suggested that the progenies of some parents performed better than others at high pH. *Vaccinium angustifolium* parents differed in the extent to which tolerance to high pH was transmitted. In vitro screening in concert with a traditional breeding program should be effective in improving blueberry tolerance to higher pH.

Highbush blueberry is typically found in sites with low pH and moist soils that are high in organic matter (Cain, 1952; Coville, 1910; Galletta, 1975; Harmer, 1944; Korcak et al., 1982). Commercial blueberry production is limited to soils with inherently low pH or that have been treated with acidifying soil amendments (Ballinger, 1966; Chandler et al., 1985). The pH range for optimal blueberry (*V. angustifolium* Ait., *V. corymbosum*) growth is between 4.5 and 5.2 (Hall et al., 1964; Harmer, 1944; Johnston, 1948; Townsend, 1966, 1967, 1969). Recognition of the acidic soil requirements, coupled with the knowledge that nutrient availability and plant metabolism are affected by changing pH levels, provided an avenue of research for understanding poor blueberry growth at higher than optimal pH levels. Initial inquiries concentrated on the availability of macronutrients, such as Ca, that were often associated with pH changes (Bailey, 1941; Cain, 1952; Harmer, 1944; Kramer and Schrader, 1942). Later investigations focused on micronutrient effects as these were being studied in many crop plants (Twyman, 1946, 1950). Iron received the greatest attention, because blueberries often exhibited the appearance of iron deficiency on higher pH soils (Bradley and Smittle, 1965; Cain, 1952, Cain and Honey, 1955; Holmes, 1960).

Although NO₃-N alone or in combination with NH₄-N is recognized as being more efficient than NH₄-N alone for the nutrition of many crops, the Ericaceous plants readily use NH₄-N (Ingestad, 1973; Townsend, 1966, 1967, 1969). Ammonium is the predominant inorganic form of N at native blueberry sites; thus, some researchers have suggested that blueberries grow

poorly on higher pH soils due to inefficient use of NO₃-N, the predominant inorganic nitrogen form at high pH levels (Cain, 1952; Havill et al., 1974; Rorison, 1986).

Over the last several years, blueberry researchers have tried to formulate an inclusive theory on blueberry intolerance of high pH soils (Korcak, 1989). Although this research has allowed the development of cultural systems that adapt the soil medium to the plant, it has not yet led to the development of cultivars adapted to variable soils.

Brown and Draper (1980), using a greenhouse solution culture screening method, identified populations, derived from *V. ashei* Reade, *V. darrowi* Camp, and *V. corymbosum*, that varied in the ability to lower the pH of nutrient solutions by releasing H⁺ ions from the roots. This process freed Fe that accumulated on roots, making it available for plant use. Progenies exhibiting this trait were termed Fe-efficient and were thought to be tolerant of high solution pH. Brown and Draper suggested that breeding programs designed to improve Fe efficiency would yield plants adapted to a wider soil pH range.

Ideally, cultivars adapted to upland mineral soils would be tolerant of lower organic matter, higher pH, and lower average moisture content than is typical of native blueberry sites (Chandler et al., 1985). Korcak et al. (1982), using broad-based germplasm derived from *V. corymbosum*, *V. ashei*, *V. darrowi*, *V. tenellum* Ait., *V. atrococcum* (Gray) Heller, and *V. angustifolium*, found variability among populations and individuals for tolerance to upland mineral soils. *Vaccinium angustifolium* appeared to be the best germplasm source for adaptation to mineral and high pH soils, based on the progenies of interspecific hybrids (Korcak, 1986a, 1986b; Korcak et al., 1982). Galletta (1975) also suggested that *V. angustifolium* maybe a source for adaptation to a broader range of physical and chemical soil characters.

Chandler et al. (1985) determined that the general combining ability (GCA) variance components were much greater than specific combining ability (SCA) components for upland soil adaptability, as measured by plant canopy volume, in progenies derived from *V. corymbosum*, *V. angustifolium*, *V. ashei*, *V. atrococco-*

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cum, *V. myrtilloides* Michx., *V. myrsinites* Lamarck, and *V. darrowii*. This result suggested that a breeding scheme designed to maximize improvement due to additive genetic variance would produce populations more adapted to upland soils.

Chandler et al. (1985) also recognized that soil variability in the field had a large effect on seedling vigor. They suggested that initial screening for upland soil adaptability be done in the greenhouse or in a place where soil conditions could be carefully controlled. Field and greenhouse screening procedures have been described to identify individuals or populations more tolerant of upland soils (Korcak, 1986a; Korcak et al., 1982) or high pH (Finn et al., 1987).

In vitro systems provide a more highly controlled environment than field or most nonfield screening environments. In vitro systems have been used in blueberry research to study propagation (Chandler and Draper, 1986; Frett and Smagula, 1983; Hall, 1976; Lyrene, 1980; Nickerson, 1978; Wolfe et al., 1983, 1986; Young and Cameron, 1985; Zimmerman and Broome, 1980) and in research of other fruit species to examine or stimulate genetic variation (Hurwitz and Agrios, 1984; Kouider et al., 1984; McPheeters and Skirvin, 1980; Navarro et al., 1985). An in vitro screening system can be closely controlled and yet be sufficiently flexible to allow nutrient or pH level adjustments. Blueberries are one of the few fruit crops that can be screened on a whole-plant basis in vitro, due to their small seedling size and relatively slow growth rate. An in vitro screening system might provide for rapid, efficient, and economic screening of seedling material for higher pH tolerance.

The objectives of this study were to: 1) study the inheritance of tolerance to higher pH in the seedlings of progenies derived from *V. angustifolium*, *V. corymbosum*, or *V. angustifolium/V. corymbosum* ancestry; 2) examine the response of blueberry plants to different in vitro pH levels, when both NO₃-N and NH₄-N forms are available in the media and the variation in iron availability is minimized; and 3) establish an in vitro screening system to identify individual seedlings or populations that exhibit higher pH tolerance.

Materials and Methods

The eight parents included representatives from *V. angustifolium*, *V. corymbosum*, and *V. corymbosum/V. angustifolium* derivatives and were crossed in a complete diallel with reciprocals (Table 1). Seeds from the 64 crosses were extracted from

Table 1. Identification and ancestry of *Vaccinium* clones used as parents in the diallel cross.

Parent ^z	Species ancestry
N70127 ^y	<i>Vaccinium angustifolium</i>
N70145 ^x	<i>V. angustifolium</i>
GR-1 ^y	<i>V. angustifolium/V. corymbosum</i>
MN-61	<i>V. corymbosum</i> (USDA 11-93) x <i>V. angustifolium</i> var. <i>nigrum</i>
Northblue	<i>V. angustifolium/V. corymbosum</i>
B-16	<i>V. corymbosum</i> (G65) x <i>V. corymbosum</i> (Ashworth)
Spartan	<i>V. corymbosum</i>
Bluejay	<i>V. corymbosum</i>

^zNumbers are Minnesota fruit accession numbers; MN, B, and GR prefixes indicate selections from the Minnesota program. G65 is a selection from the U.S. Dept. of Agriculture breeding program.

^yClone resulting from open-pollination of a *V. angustifolium/V. corymbosum* derivative of unknown origin growing in the Harvard Forest (Massachusetts); selected at Grand Rapids, Minn.

^xCollected from a wild stand in northern Minnesota.

fresh fruit, sterilized for 5 min with diluted commercial bleach (20%), rinsed with sterilized distilled water, and planted on an agar nutrient medium in glass jars (6 cm diameter x 10 cm height, 30 ml medium per jar). Twenty seeds were planted in each jar. A modified Zimmerman's medium (Zimmerman and Broome, 1980) was used of the following composition: (mM), NH₄NO₃, 0.4; (NH₄)₂SO₄, 0.3; Ca(NO₃)₂·4H₂O, 0.6; KNO₃, 0.4; KH₂PO₄, 0.6; MgSO₄·7H₂O, 0.3; FeSO₄·7H₂O, 0.2; Na₂EDTA, 0.2; MnSO₄·H₂O, 0.1; H₃BO₃, 0.1; (μM), KI, 5.0; Na₂MoO₄·2H₂O, 1.0; CoCl₂·H₂O, 0.1; CuSO₄·5H₂O, 0.1. Agar was added to the medium at 5.5 g-liter⁻¹. The pH was adjusted with NaOH to either 5.0 or 6.0. The lower pH was chosen because it falls within the pH range where optimal blueberry growth occurs in soils and it was above the range where agar solidification can be a problem. The higher pH was chosen because, in preliminary experiments at pH 6.0, the plants exhibited a stress response and growth differences could be observed among plants. Succinic acid (0.01 M; pK_a at 25C = 4.19 and 5.57) and Mes, (0.02 M; pK_a at 25C = 6.15) were used to maintain the initial pH levels over the course of the study. Jars were capped, loosely sealed with Parafilm, and placed in a germination room under fluorescent lights [photosynthetic photon flux density (PPFD) = 30 μmol·s⁻¹·m⁻², 30 cm from light] that were programmed for a 14-hr light and 10-hr dark period. The chamber was maintained at 22C and 65% RH.

The pH of the bulk medium was measured at the end of the experiment with a Corning combination (#476115) surface probe. Rhizosphere pH was measured using a microelectrode [(MEPH-1), World Precision Instruments; New Haven, Conn.] by inserting the probe to within 2 mm of the root surface.

Plants were grown for 21 weeks. At the conclusion of the experiment, data were collected on plant survival and growth traits described in Table 2. Analyses of variance were computed for a completely randomized design. One self-pollinated population failed to produce seed; therefore, only 63 populations were analyzed. A modified Griffing's Method 3 model (Griffing, 1956) was used to analyze the set of populations without the self-pollinated crosses. The parental effects were considered fixed variables. The GCA, SCA, maternal, and reciprocal var-

Table 2. Survival and plant growth traits evaluated on a per-jar basis for *Vaccinium* progenies grown in vitro.

Trait	Description
Survival traits (%)	
Germination	[1 - (No. of seeds that did not produce a radicle/20)] x 100
Radicle emergence	(No. of seeds that produced a radicle and grew no further/20) x 100.
Cotyledon emergence	(No. of seeds that produced both a radicle and cotyledon before senescing/20) x 100.
Survival	(No. of plants that survived until the experiment's conclusion/20) x 100.
Plant growth traits	
Vitality	Overall vitality of the progeny (1 = chlorotic and/or weak, 9 = green, healthy, and vigorous).
Ht	Total ht (mm) of all surviving plants per no. of surviving plants.
Dry wt	Total dry wt (g) (roots and shoots) of all surviving plants per no. of surviving plants.

iance components, along with the GCA and maternal effects, were estimated.

Results and Discussion

Most of the seeds germinated in 2 to 3 weeks. Very few plants died after producing true leaves. Many plants had grown to the top of the jars (≈ 10 cm) when the experiment was terminated. Although plant growth was adequate for the purposes of this study, a light source with a higher PPFD might enhance growth rates and, thus, shorten the screening period. The plants at pH 6.0 looked nearly as tall as those at pH 5.0, but they were more chlorotic and had smaller leaves. Some individuals and populations under both regimes appeared more vigorous and/or less chlorotic, suggesting that visual selection of superior seedlings was possible both within and between populations. These growth differences due to pH effects paralleled observations with *in vitro* growth of 'Bluecrop' cuttings by Wolfe et al. (1986).

Since blueberry plants in solution (Brown and Draper, 1980) or in medium (Wolfe et al., 1986) release H^+ ions that can lower the pH of the medium, maintenance of the pH levels was a concern. However, within each pH regime, the pH of the general medium and the pH in the immediate rhizosphere were very similar at the end of the experiment, indicating that the two buffers were effective in maintaining the initial pH levels (± 0.1 pH unit).

Interpopulation variation was significant, regardless of pH regime, for all of the traits examined (Table 3). Percent radicle emergence and plant survival varied little due to pH regime. A significant ($P \leq 0.05$) difference in germination was detected between the pH levels, although differences were small (78% at pH = 5.0; 75% at pH = 6.0). Significant variation due to pH regime for vitality and dry weight, but not for height, agreed with the visual observations that plants grown at higher pH were about the same height but had smaller leaves and were less vigorous when compared with the plants at low pH (Table 3). The population $\times$ pH interaction measured the variation due to differential population responses to pH and should indicate the presence of higher pH tolerance. The population $\times$ pH interaction was significant for average plant height but not for average dry weight. The populations also showed a differential response due to pH for the subjective vitality score.

The GCA variance components were significant for all of the traits measured (Table 3). The SCA variance components were significant for percent germination, radicle emergence, and survival, but for neither percent cotyledon emergence nor plant

growth evaluation traits (Table 3). The GCA mean square was similar in magnitude to the SCA mean square for percent radicle emergence, but both were much larger (3- to 26-fold) than the SCA mean squares for all other traits. Therefore, additive genetic variance appears to be more important than nonadditive genetic variance for these traits and within this germplasm.

The variance components due to maternal and reciprocal effects might be expected to be quite important in the traits involved in seed germination and initial seedling survival because the maternal parent provides the environment for seed development and maturation. However, the variance components due to maternal effects were not significant for any of the seed germination and survival traits (Table 3). Significant maternal effects for the plant growth measurements suggested that careful selection of the maternal parent may aid the development of higher pH-tolerant blueberries.

In general, the variance components due to reciprocal effects were similar in size to the maternal effects for the survival evaluation traits (Table 3), and, likewise, they were not significant. The variation due to crossing in one direction or another appears to be largely due to choice of maternal parent for these survival traits. Although nonsignificant, this trend was not surprising, because maternal effects would be expected to have a larger role in early plant development. However, the maternal components of variance were significant and two to three times larger than the reciprocal components of variance for the plant growth traits (Table 3).

The parental GCA effects for percent germination and survival were similar at each pH level, which was not surprising, based on the lack of significant pH regime effects for these traits. The parental GCA effects for percent germination were large and negative for MN-61 and 'Bluejay', whereas 'North-blue', GR-1, and N70145 consistently produced populations that had a higher percent germination (Table 4). The GCA effects for percent survival were large and negative for MN-61 and 'Bluejay', and large and positive for GR-1. Progenies derived from MN-61 and 'Bluejay' not only had poorer germination rates, but many of the seedlings died before or soon after cotyledon emergence. However, progenies of GR-1 not only had higher germination rates than the others, but very few of the plants that germinated subsequently died. While the parental GCA effects for the seedling emergence and survival traits were not associated with species background, GCA effects for the plant growth traits, population vitality, average height, and average dry weight were (Table 4). N70127 and N70145 at low and high pH had large positive effects for plant height. N70145

Table 3. Analysis of variance for survival evaluation traits for blueberry populations grown under low or high pH regimes *in vitro*.

Source	df	Mean squares						
		Germination (%)	Radicle emergence (%)	Cotyledon emergence (%)	Survival (%)	Plant vitality ^z	Plant ht	Plant dry wt
Population	62	8.77**	17.35**	25.85**	7.66**	8.98**	364.33**	0.0014**
GCA	7	21.03**	24.25**	73.85**	15.77**	15.19**	1916.11**	0.0043**
SCA	20	4.25*	21.35**	18.00	5.62**	2.25	73.73	0.0005
Reciprocal	28	3.12	6.00	15.80	2.50	4.34*	162.81*	0.0013**
Maternal	7	2.70	5.95	22.25	2.25	9.28*	450.68**	0.0039**
pH regime	1	6.20*	3.15	141.10**	0.15	26.27**	96.94	0.0039**
pH reg. $\times$ pop.	62	1.30	13.35**	19.40	2.13	2.61**	112.76*	0.0006

^zVitality measured on 1-9 scale; 1 = chlorotic and/or weak, 9 = green, healthy, and vigorous.

*,**F value > 1 ($P \leq 0.05$ and 0.01 , respectively).

Table 4. General combining ability effects for *Vaccinium* parents for survival evaluation and plant growth traits under each pH regime (low = pH 5.0, high = pH 6.0).

Parents	Germination (%)		Survival (%)		Plant vitality		Plant ht		Plant dry wt	
	Low pH	High pH	Low pH	High pH	Low pH	High pH	Low pH	High pH	Low pH	High pH
N70127	-1.95	0.27	-0.70	3.43	1.17	0.57	11.90	6.49	0.60	0.24
N70145	3.04	6.05	1.01	3.51	0.95	0.32	6.70	6.57	0.15	-0.05
GR-1	5.49	6.10	4.35	3.80	0.20	0.20	-0.41	2.00	0.07	0.27
MN-61	-9.77	-9.42	-8.36	-12.50	-0.64	-0.87	-5.66	-2.47	-0.18	-0.26
Northblue	2.27	7.77	3.35	4.06	0.04	0.42	-2.44	-0.23	-0.03	0.17
B-16	-1.02	-1.92	4.76	1.79	-1.05	-0.77	-7.85	-7.76	-0.51	-0.39
Spartan	5.40	1.83	-2.11	2.18	-0.77	0.14	-2.53	-0.49	-0.16	-0.08
Bluejay	-3.46	-10.68	-2.30	-6.26	0.10	-0.01	0.28	-4.12	0.06	0.11
SE ²	2.65	2.57	2.97	2.91	0.28	0.27	1.87	1.48	0.09	0.12

²Mean standard error of parental effects.

at low pH and N70127 at high and low pH had positive effects for vitality. GR-1 at high pH and N70127 at low and high pH had large and positive effects for dry weight. The parents with *V. angustifolium* or species hybrid ancestry, except MN-61, contributed to more vigorous populations with higher dry weights (Tables 1 and 4). Those parents within this germplasm with 100% *V. angustifolium* ancestry strongly contributed to taller plant height, regardless of pH level. The progenies of species hybrid derivative or *V. corymbosum* parents tended to be shorter than the rest, regardless of pH level. 'Bluejay', which was largely neutral for dry weight, height, and vitality GCA effects at both pH levels, had a very high negative effect for plant height at high pH. B-16 (*V. corymbosum*) and MN-61 (species-hybrid derivative) had large and negative GCA effects for vitality, height, and dry weight at both pH levels.

The maternal effects were largely nonsignificant (less than 2× SE), with a few exceptions (data not shown). The populations with N70145 as the maternal parent had high incidence of seed germination. However, this effect did not contribute to a higher maternal percent survival effect. B-16 had a significant and negative maternal effect for plant vitality (-6.26), height (-48.62 mm), and dry weight (-3.01 mg). The combination of poor GCA effects and maternal effects suggested that B-16 would be a poor choice as a parent to enhance high pH tolerance. 'Northblue' and 'Bluejay' both had significant and positive maternal effects for plant vitality (3.81 and 5.25, respectively), height (23.3 and 36.6 mm, respectively), and dry weight (2.22 and 3.93 g, respectively). Within the restrictions of this study, these parents could be considered good maternal parents, although their overall GCA effect did not suggest that they are outstanding parents for transmitting higher pH tolerance.

Phenotypic correlation coefficients between height and dry weight were positive and high over both regimes and within the low pH regime, and positive but lower within the higher pH regime (Table 5). The vitality scores were consistently more positively correlated with plant height than dry weight, suggesting that when these cultures are subjectively evaluated, height is given more value than true vitality. Although height was more highly correlated than vitality scores with dry weight at low pH, at high pH they were equal.

In general, the progenies with greater percentage lowbush ancestry were taller, had greater dry weight, and higher vitality scores than those with a larger percentage of highbush ancestry (Table 5). This trend was most noticeable for plant height in

Table 5. Correlation coefficients among plant vitality, height, and dry weight (family means over pH regimes and within each pH regime).^{2,3}

Plant character	Ht	Dry wt	Lowbush ancestry (%)
Vitality-overall	0.73	0.63	0.39
pH 5.0	0.79	0.65	0.51
pH 6.0	0.67	0.58	0.27 (0.03)
Ht-overall		0.73	0.54
pH 5.0		0.85	0.53
pH 6.0		0.59	0.56
Dry wt-overall			0.26
pH 5.0			0.37
pH 6.0			0.15 ^{NS}

²The three correlations are for either over pH regimes or within the low or high pH regime.

³ $r \neq 0$ ($P \leq 0.01$; df = 128 overall and 64 within each regime), unless otherwise indicated.

^{NS}Nonsignificant.

general and at low pH for vitality and dry weight. Plant height was positively correlated with percent lowbush ancestry at each pH level. The confines of this in vitro system did not allow a determination of when the largely highbush progenies would overtake the largely lowbush progenies as measured by height.

Media pH affected plant growth despite the availability of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ forms and the increased availability of Fe with EDTA chelate. Some clarification of this statement is necessary, however. Although concentrations of Fe and other nutrients were initially the same, varying pH levels can affect their activity in solution (agar). A modified speciation model (GEO-CHEM; Parker et al., 1987) was used to calculate activity of Fe^{3+} in the nutrient solutions adjusted to a pH of 5 and 6. The results indicated that Fe^{3+} activity was 66 times greater at pH 5 compared to pH 6. To overcome this problem, future experiments should include buffering the Fe with excess chelate so that pH and Fe effects are not confounded (Chancy et al., 1989).

Little variation was observed for tolerance of higher pH as measured by plant dry weight within the germplasm examined in this study. However, the parental GCA effects suggested that the progenies of some parents performed better than others at high pH levels. There are at least two possible reasons GCA effects suggested higher pH tolerance even though the population × pH regime interaction was not significant for dry weight:

1) populations that were the most vigorous at high pH also were better at low pH, or 2) the interaction was masked by the poorer overall performance of the populations derived from B-16 and MN-61. The first alternative may not be appropriate because the progenies of GR-1 grew better than many others at high pH but were similar to other populations at low pH. Progenies of GR-1 were also considered more tolerant of high pH in a field study where 2-year-old plants were evaluated (Finn, 1989).

In general, the populations derived from parents that had a large proportion of *V. angustifolium* ancestry, especially those of N70127, performed better than others at high pH and overall, but the progenies of MN-61 (50% lowbush) were significant exceptions. Although others have proposed that *V. angustifolium* could be a general source of genes for tolerance to high pH (Galletta, 1975; Korcak, 1986a, 1986b; Korcak et al., 1982), our data suggest that individual *V. angustifolium* genotypes differed to the extent that some pass increased tolerance to high pH to progeny and others do not. Therefore, careful selection of parental material is critical.

In vitro screening in concert with a traditional breeding program should be effective in improving blueberry tolerance to high pH in a manner similar to the Fe-efficiency screening procedure of Brown and Draper (1980). Based on the importance of GCA variance within this germplasm, a breeding system using recurrent, phenotypic (mass) selection to obtain genotypes with greater pH tolerance in vitro should be successful.

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